University of Alberta

The effects of barley-derived β-glucan on intestinal permeability and morphology in BB rats

by

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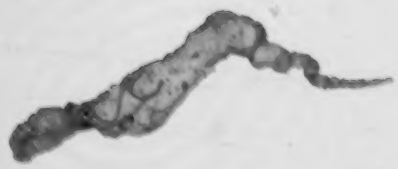

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Abstract

This study evaluated the effects of adding β-glucan of different sources and viscosities on intestinal permeability and morphology in rats. Young BioBreeding normal (BBn) and –diabetes-prone (BBdp) rats were randomized to 1 of 5 diets differing only in fibre composition: cellulose, high viscosity β-glucan (HVβG), low viscosity β-glucan (LVβG), barley flour, or oatmeal. BBn rats fed the LVβG diet had increased permeability of the ileum ($p=0.004$) but not the jejunum, compared with those fed the barley, oatmeal, or cellulose diets. Ileal permeability was positively correlated ($r=0.515$, $p<0.001$) with the amount of β-glucan. Diet had no effect on electrophysiological parameters, villus height, crypt depth, and number of IgA+ cells. In BBdp rats, permeability, villus height, crypt depth, and number of IgA+ cells were not affected by diet. There were no differences between HVβG and LVβG groups in both strains of rats. In conclusion, the diet containing LVβG increased ileal permeability without changing the morphology or activating the formation of IgA+ cells.

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List of abbreviations

A.O.A.C	Association of Official Agricultural Chemist
Ab	Antibody
ABC	Avidin Biotinylated Enzyme Complex
ANOVA	Analysis of Variance
BBdp	BioBreeding diabetes-prone
BBn	BioBreeding normal
Ca	Calcium
cAMP	Cyclic andenosine monophosphate
CDC	Chenodeoxycholic Acid
CVD	Cardiovascular Disease
DAB	DiamnoBenzine Tetrahydrochloride
dH_20	Distilled Water
E.coli	*Escherichia coli*
ETOH	Ethanol
FOS	Fructooligosaccharides
G	Conductance
GALT	Gut Associated Lymphoid Tissue
GBF	Germinated Barley Foodstuff
GI	Gastrointestinal
GLP-1	Glucagon-like Peptide-1
GLP-2	Glucagon-like peptide-2
H&E	Hematoxylin & Eosin
HDL-C	High Density Lipoprotein Cholesterol
HEC	Hydroxyethyl Cellulose
HVβG	High Viscosity β-glucan
LVβG	Low Viscosity β-glucan
IEL	Intraepithelial lymphocyte
IFN-γ	Interferon-gama
IgA	Immunoglobulin A
IL	Interleukin
I_{SC}	Short Circuit Current
IV	Independent Variable
KRH	Krebs-Ringers-Hepes
LDL-C	Low Density Lipoprotein Cholesterol
LP	Lamina propria
Mg	Magnesium
MLN	Mesenteric Lymph Nodes
mRNA	Messenger RNA
MUFA	Monounsaturated Fatty Acid
P:S	Polyunsaturated : Saturated fatty acid ratio
PBS	Phosphate Buffered Saline

PD	Potential Difference
PEG	Polyethylene Glycol
PP	Peyer's Patches
PSP	Phenolsulfonphthalein
PTM	Protamine
PUFA	Polyunsaturated Fatty Acid
SCFA	Short Chain Fatty Acid
TAP	2,4,6, Traminopyrimidine
TIU	Trypsin inhibtor unit
TNF-α	Tumor Necrosis Factor alpha
TPN	Total Parenteral Nutrition
UWL	Unstirred Water Layer
Zn	Zinc

Chapter 1

Literature Review

1.1. Introduction

Nondigestible carbohydrates in our diets, often referred to as dietary fibre, have numerous effects on the intestine (Dongowski et al., 2002;Dongowski et al., 2003) the immune system (Schley and Field, 2002), and in the prevention of certain cancers (Mattisson et al., 2004). Research on fibre has primarily focused on its roles in diverticular disease, colon cancers, glucose absorption, and lipid metabolism (McPherson, 1993). However, less attention has focused on the specific effects of fibre on the barrier function and morphology of the intestine. This study was conducted to evaluate the effects of different types (oats versus barley and whole grain barley versus purified barley β-glucan extracts) and viscosities (high and low) of β-glucan on small intestinal permeability, morphology, and the number of immunoglobulin A (IgA) positive cells (see appendix 1). This chapter will cover the definition of fibre, its general effects on health, the structure and function of the intestines in health and disease, the intestinal immune system, the effect of fibre on the intestine, and the definition and role of β-glucan.

1.2. Definition of "fibre"

Discussing "fibre" and its health benefits requires first discussing what is meant by "fibre." There have been numerous definitions for "fibre," which were primarily based on analytical methods and followed a common theme that "fibre" is resistant to

digestion by enzymes in the human gastrointestinal (GI) tract. In the early 20th century, a harsh extraction process was used to determine the crude fibre content of foods (Spiller, 1993). After determining that 50-85% of "fibre" was destroyed in the process of determining crude fibre, the term dietary fibre replaced crude fibre (Trowell, 1985;Trowell et al., 1985) and the definition was changed to refer to materials derived from plant cell walls (Trowell et al., 1985). Over approximately 15 years, the definition was modified to include both carbohydrates from the storage compartment and the cell walls of plants and to distinguish between the two sources (Southgate, 1977;Trowell et al., 1985). Debate also rose regarding whether nondigestible materials from other plant sources (e.g. waxes, silica, cutins, cell wall proteins) and animal sources (e.g. chitin, chitosan, aminopectylin) should be included in the definition of dietary "fibre" (Trowell et al., 1985;A report on the definition of dietary fiber, 2001;McPherson, 1993). Over time, other names for dietary "fibre" (e.g. plantix, unavailable carbohydrate, neutral detergent residue, non-starch polysaccharide, soluble and insoluble fibre) were also proposed (Spiller, 1993;Briggs and Spiller, 1977). Furthermore, because of recent food labeling and the potential inclusion of health claims on products, the definition of "fibre" was broadened to include biochemical and health effects.

In 1985, Health Canada accepted a definition of dietary "fibre" as the "endogenous components of plant materials in the diet that are resistant to digestion by enzymes produced by humans. They are predominantly non-starch polysaccharides and lignin and may include, in addition, associated substances"(Health and Welfare Canada, 1985). In 2001, the Dietary Reference Intake panel (A report on the definition of dietary fiber, 2001) was mandated to propose an accurate definition that took into account

analytical methods and physiological effects and which would facilitate the comparisons among studies and comprehension in the field of nondigestible carbohydrates (A report on the definition of dietary fiber, 2001). Three categories of fibre were proposed: first, *dietary fibre* which represents the "nondigestible carbohydrates and lignin that are intrinsic and intact in plants"(A report on the definition of dietary fiber, 2001). These materials are integral to plant structure. Cereal brans with intact cells and which have starch and proteins are an example of *dietary fibre*, as well as corn cereal, raffinose, and low molecular weight fructans found in foods (A report on the definition of dietary fiber, 2001). Second, *functional fibre* is the "isolated, nondigestible carbohydrates that have beneficial physiological effects in humans" (Food and Nutrition Board, Institute of Medicine, 2002). These fibres may be isolated, extracted, or manufactured. Pectin isolated from citrus peel and β-glucan extracted from barley or oats are examples of *functional fibre*. Third, *total fibre* is the sum of the previous two categories. Using the DRI proposed definition, β-glucan derived from barley is considered to be a *functional fibre* and barley flour, oatmeal, and cellulose are sources of *dietary fibre* (A report on the definition of dietary fiber, 2001). For the purpose of this thesis, the fibre terms proposed by the DRI will be used unless otherwise specified. Also, the term used in published papers will be kept when describing the study, which does not necessarily reflect the DRI definition.

1.3. Overview of fibre and health

Total fibre derived from consuming a variety of foods may directly or indirectly contribute to satiety, health, and energy. The average intake of total fibre in North

America ranges from 12.1 to 17.9g per day for females and males 19 years of age and older (Food and Nutrition Board, Institute of Medicine, 2002). The important role of fibre on health was hypothesized, a few decades ago, based on ecological studies in which populations in Africa experienced fewer chronic diseases in the hunter gatherer lifestyles compared to Western civilizations with low fibre consumption (reviewed by Burkitt, 1993;Trowell, 1985). Today, fibre consumption has been suggested as part of the treatment and management of diabetes (Li et al., 2003a;Anderson et al., 2004;Franz et al., 2002), dyslipidemia, (Jenkins et al., 2002b;Jenkins et al., 2003), diverticular disease (American Dietetic Association and Dietitians of Canada, 2000), and constipation (Morais et al., 1999). Fibre has been shown beneficial by attenuating the postprandial rise in blood glucose concentrations (Jenkins et al., 1977;Jenkins et al., 2002a;Braaten et al., 1994a;Wursch and PiSunyer, 1997), reducing postprandial insulin concentrations (Braaten et al., 1994d;Vachon et al., 1988), lowering the absorption of carbohydrates (Lifschitz et al., 2002), and improving long-term glycemic control (i.e. hemoglobin A1c) (Li et al., 2003b). Furthermore, viscous fibres in the diets of people have been shown to slow the absorption of glucose (Jenkins et al., 2000a; Jenkins et al., 2000b;Flourie et al., 1984) and when added to a glucose solution, viscous fibres reduce the *in vitro* diffusion of glucose (Ou et al., 2001).

The low density lipoprotein cholesterol (LDL-C) to high density lipoprotein cholesterol (HDL-C) ratio has been shown to improve with the consumption of fibre and a low fat diet (Mckay and Ball, 1992). Viscous fibres have also been shown to reduce total cholesterol and LDL-C in hypercholesterolemic people (Mcintosh et al., 1991;Braaten et al., 1994b;Brown et al., 1999;Jenkins et al., 2003) in many, but not all,

studies (Torronen et al., 1992). Discrepancies in the literature may reflect differences in the diet preparation methods and the resulting structure of the fibre (Torronen et al., 1992). Therefore, the type of fibre, its source, and its viscosity may have an integral role on the physiological effect of the fibre.

1.4. Intestinal structure

The GI tract is an extension of the external environment that stretches from the mouth to the anus. The small intestine is located approximately mid way through the GI tract and consists of the duodenum, where some digestion and absorption of nutrients occurs, the jejunum, where the majority of digestion and absorption happens, and the ileum, the segment before the large intestine and primary site of water absorption in the small intestine. The mucosa of the intestines has convoluted folds and on these folds are villi that increase the surface area (Rubin, 2003). The villi in the proximal small intestine (i.e. jejunum) have a finger-like structure and are more numerous compared to the ileum or distal small intestine which have shorter and fewer villi (Madara and Anderson, 2003). Crypts, located at the bottom of the villi, are sites of cell proliferation (Shiner, 1994) and secrete anti-microbial substances (e.g. defensin) (Tomas, 2004). On the surface of the villi, the absorptive enterocytes are interspersed with mucus-producing goblet cells and intraepithelial lymphocytes (IEL). Enterocytes are connected to each other via tight junctions, which can be used as a low resistance shunt for paracellular diffusion of molecules (Madara and Anderson, 2003). Central to the epithelial structure and gut immune system is the lamina propria (LP), which consists of blood vessels, immune cells (e.g. lymphocytes, plasma cells, eosinophils), nerves, and muscles (Rubin, 2003). An important part of the GI tract is the gut associated lymphoid tissue (GALT). GALT is

composed of mesenteric lymph nodes (MLN), IEL, LP, Peyer's Patches (PP) and M cells that cover the PP. Peyer's Patches have T-cells, B-cells, and plasma cells (Brandtzaeg et al., 1985;Madara and Anderson, 2003). The number of PP is increased in the ileum compared with the duodenum or jejunum. These PPs are involved in sampling the luminal contents, processing, and presenting the antigens to T or B-cells. When a perceived threat (e.g. antigen) is identified during the M cells and PP sampling of the luminal contents, a signal is sent to certain plasma cells to secrete immunoglobulin A (IgA) (Brandtzaeg, 1988;Brandtzaeg et al., 1985).

1.5. Functions of the small intestine

The GI tract is involved with transporting, digesting, and absorbing nutrients and foods that are ingested and it is involved in excreting non-digestible food components. The intestinal barrier must allow nutrients to be absorbed while it protects the body from pathogens or potentially harmful dietary antigens (Söderholm, 1998). A healthy intestinal barrier is composed of several physiologically important layers. The unstirred water layer (UWL) which lies above the mucus membrane facing the lumen may reduce the absorption of nutrients (Farhadi et al., 2003;Madara and Anderson, 2003;Thomson, 1983) and may contribute to the barrier function of the intestine (Thomson, 1983). Below the UWL is the mucous membrane that covers the epithelial cells and protects it from physical damage (Farhadi et al., 2003) and adherence of bacteria to the epithelia (Madara and Anderson, 2003). The epithelial cells serve as a site of absorption and passage of macromolecules (Söderholm, 1998). These epithelial cells are joined by tight junctions that give a continuous structure and sites for diffusion of some solutes (Farhadi et al.,

2003;Söderholm, 1998). Tight junctions are a site that may be altered by a lack of oral food intake or diseases (e.g. crohn's and celiac) which may affect the intestinal barrier function (Bruewer et al., 2003;Söderholm, 1998). A thorough discussion on tight junctions is reviewed by Gonzalez-Mariscal et al., (2003). The immune system consisting of macrophages, lymphocytes, intraepithelial lymphocytes (IEL), and immunoglobulin that are located in the LP and between enterocytes (Farhadi et al., 2003). Immunoglobulin A (IgA) is secreted into the lumen by plasma cells in the LP to help bind virus or bacteria before they adhere to the intestinal epithelium (Spaeth et al., 1994) and translocate to the MLN (Mosenthal et al., 2002). IgA increases the secretion of mucus which limits the passage of soluble antigens through the enterocytes. Together these layers and structures form the intestinal barrier.

A compromised barrier is associated with diseases affecting the intestine and the overall health of the person. Increased intestinal permeability (i.e. reduction in barrier function) has been observed prior to the recurrence of inflammation in Crohn's disease (Söderholm, 1998), prior to the onset of celiac disease (Smecuol et al., 1997), and to type 1 diabetes (Carratu et al., 1999;Meddings et al., 1999). The integrity of the barrier function can be decreased by immunosuppressive drugs such as rapamycin, cyclosporine A (Dias et al., 1998), and FK506 tacrolimus (Madsen et al., 1995a;Madsen et al., 1995b), cytokines including tumor necrosis factor (TNF-α) (Bruewer et al., 2003), interferon-gamma (IFN-y) (Bruewer et al., 2003;Yang et al., 2003), and interleukin (IL-4) (Madden et al., 2002;Colgan et al., 1994), bile acids (Rehman et al., 2003;Teichberg et al., 1983), fasting, and total parental nutrition (TPN) (Deng et al., 1999;Ferraris and Carey, 2000;Mosenthal et al., 2002;Ohta et al., 2003;Omura et al., 2000). Dietary fibre has been

shown to improve barrier function (Deng et al., 1999;Mosenthal et al., 2002) and reduce the movement of molecules through the paracellular route in the small and large intestines (Mariadason et al., 1999;Rehman et al., 2003). The effects of fibre on intestinal barrier function will be further discussed in a later section 1.7.

1.5.1. Measurements of intestinal barrier function

Alterations in barrier function are assessed by permeability measurements. There are *in vivo* and *in vitro* methods of assessing intestinal permeability as well as indirect methods such as measuring bacterial translocation to MLN, spleen or liver (Farhadi et al., 2003). One set of methods used to determine intestinal permeability in humans or animals consists of quantifying the recovery of ^{51}Cr-EDTA, polyethylene glycol (PEG) 4000 or sugars (e.g. sucrose, lactulose, mannitol, rhamanose, cellubiose, and sucralose used alone or in combination) in urine (Bjarnason, 1994;Farhadi et al., 2003). The recovery of sucrose correlates with increased permeability of the stomach and the duodenum (Farhadi et al., 2003). The recovery of lactulose, mannitol or rhamanose reflects an overall increase in the permeability of the small intestinal (Farhadi et al., 2003f). The lactulose: mannitol test technique has been validated to detect permeability changes (Wring et al., 1998) and is used as a screening tool to identify patients that required more invasive GI testing (Di Leo et al., 2003). These *in vivo* techniques can be useful in the clinical setting where the patients needs assessment on GI function (Di Leo et al., 2003) without removing a section of the GI tract. However, these methods cannot be used to assess the electrophysiological parameters that reflect ion transport.

There are several *in vitro* techniques for measuring permeability. These include measuring the flux of a probe through a monolayer of intestinal cells (Farhadi et al., 2003) or through tissues using the Ussing chamber (Ussing and Zerahn, 1951;Söderholm, 1998). Such methods assess ex vivo barrier function of the monolayer or tissue. By using probes such as phenolsulfonphthalein (PSP) (Ohta et al., 2003;Omura et al., 2000) and measuring its flux across a monolayer, one can assess the transepithelial resistance or the ability of the cells to resist movement of molecules.

The Ussing chambers method assesses permeability of the tissue or mucosal sheet, in an environment that mimics the body, while also measuring the electrophysiological characteristics of the tissue. For this method, an intestinal section is mounted in the Ussing chamber, which is connected to two reservoirs filled with a physiological buffer that bathes both the luminal and serosal sides of the tissue. The buffer is maintained at 37°C and bubbled with 95% O_2 and 5% CO_2. Two voltage electrodes are attached to the back of the chamber near the tissue, and two current electrodes are attached to the front of the chamber. These electrodes measure the electrophysiological state of the tissue (Mishima et al., 1999).

The electrophysiological characteristics that are measured are short circuit current (I_{sc}), potential difference (PD), and resistance (R). I_{sc} represents total bidirectional ion movement (Steward and Case, 1989). PD reflects the tissue's ability to maintain the ion concentration gradient across the intestinal tissue (Grotz et al., 1999), which is the related to the tissue's ability to carry out active transport (Steward and Case, 1989). PD is mainly driven by sodium flux (Ussing and Zerahn, 1951). The third measurement, R,

indicates the integrity of the tissue (Grotz et al., 1999); and the inverse of resistance is termed conductance (G).

Currently, there are no valid *in vivo* techniques that would measure precisely the permeability area and electrophysiological characteristics of the intestine. Therefore, the drawback of this *in vitro* technique is that the animal is sacrificed to remove of the intestine to conduct detailed permeability measurements. For the thesis project, the *in vitro* Ussing chamber method was used in order to assess the permeability along with the ion status of the tissue.

1.5.2. The intestinal barrier in disease states

The intestinal barrier is compromised in several diseases such as celiac disease (Farhadi et al., 2003;Smecuol et al., 1997), Crohn's disease (Söderholm, 1998;Thomson et al., 1997), and type 1 diabetes (Carratu et al., 1999;Hardin et al., 2002;Meddings et al., 1999). A reduction in the UWL, physical damage to the mucosa, disruption of tight junctions, and activation of the inflammatory responses may contribute to the decreased intestinal barrier observed in some diseases. For example, a reduction in the UWL was seen in rats with streptozotocin-induced diabetes (Thomson, 1983). Physical damage to the mucosa (e.g. villi atrophy) and damage to the tight junctions have been reported in Crohn's disease (Söderholm, 1998). Zonulin, a tight junction protein, has been reported to be damaged in celiac disease, possibly due to exposure to the gliadin protein, a proposed antigen in the pathogenesis of celiac disease (Clemente et al., 2003). All of these factors contribute to reducing the ability of the intestine to exclude pathogens and potentially harmful antigens (Söderholm, 1998). Once the harmful antigen crosses

through the epithelium and reaches the immune system in the lamina propria, the local immune system can be activated (Farhadi et al., 2003). Activation of the local immune system may invoke an inflammatory response and produce proinflammatory cytokines such as IFN-γ and TNF-α (Bruewer et al., 2003). This then disrupts tight junctions and further activates the immune system.

Inflammation may activate the complement system, causing an increase in the production of IgG antibodies. These processes may damage the epithelia and allow more antigens to penetrate the intestine. Processes that reduce the barrier function of the intestine may be perpetuated since once the barrier is breached, more antigens are able to penetrate the mucosa and stimulate the immune system, which further compromises the intestinal barrier function.

1.6. The intestinal immune system

The gut associated lymphoid tissue (GALT) is one of the largest immunological tissues in the body. It protects the intestine from pathogens and processes antigens to cause tolerance or an immune reaction to defend the intestine and the host (Langkamp-Henken et al., 1992). The GALT is composed of M cells, PP, LP with lymphocytes, macrophages, plasma cells, and the MLN (Schley and Field, 2002;Langkamp-Henken et al., 1992). M cells protrude into the intestinal lumen and are involved in the process of sampling luminal contents. Antigens are transported to the PP where they are processed by antigen presenting cells (e.g. macrophages, dendritic cells) and presented to T- or B-cells (Langkamp-Henken et al., 1992). T-cells in the PP enhance IgA production while suppressing the IgM and IgG (Langkamp-Henken et al., 1992).

One product of B-cells in the gut is IgA. The production of IgA specific B-cells requires that cells leave the PP through the MLN, pass through the thoracic duct to enter the systemic circulation (Langkamp-Henken et al., 1992) and eventually home back to the LP (Brandtzaeg et al., 1985). In the LP, B-cells finish maturing and become plasma cells capable of secreting IgA (Brandtzaeg, 1988). The secretory component is synthesized and attached to the IgA (Brandtzaeg, 1988) for its release into the lumen when the intestine needs protection against a particular pathogen. IgA is important because it serves as an antimicrobial agent (Langkamp-Henken et al., 1992) by preventing the binding of bacteria or viruses to the intestinal epithelium (Schley and Field, 2002;Brandtzaeg et al., 1985;Spaeth et al., 1994). IgA also reduces the ability of soluble antigens to penetrate the intestinal barrier (Brandtzaeg, 1988).

1.6.1. The effect of fibre on the GALT

Total fibre has been suggested to affect the GALT and other cell-types in the intestine. An extensive review of the effects of fibre on GALT was compiled by Schley and Field, (2002) and reaches beyond the scope of this thesis. However, a key idea examined by Schley and Field, (2002) that is directly related to this thesis project is that fibre has an overall effect on the intestine and GALT. They concluded that fermentable fibre may alter the phenotype and function of the GALT.

Total fibre may affect phenotype switching in the GALT. Field et al (1999) found that switching the diet from one that is low to one that is high (8.7g/kg) in fermentable fibre (oligofructose, gum arabic, and beet pulp, combined) caused an increased percentage of CD8 T-cells in the LP, IEL, and PP and caused an increased proportion of

CD4 T-cells in the MLN in dogs (Field et al., 1999). Lim et al, 1997 found an increased fraction of CD4 T-cells in the MLN of rats whose water contained soluble fibre (i.e. pectin, konjak mannan) compared to rats fed cellulose or the acid-soluble chitosan as the fibre source. Feeding diets containing 5% (w/w) pectin to rats increased the concentration of IgA in the MLN and in the serum (Lim et al., 1997).

Other researchers evaluated the effect of fibre and its fermented by-products on the function of the immune system and the intestine. Rodriguez-Cabezas et al., (2003) evaluated the effect of Plantago ovata, a fermentable fibre from which psyllium is derived, on the production of SCFA and inflammation in the colon. They found that adding fibre to the diet (5g in 95g diet) increased the production of butyrate and proprionate (Rodriguez-Cabezas et al., 2003). This increase in SCFA was associated with a reduction in the proinflammatory mediators: TNF-α, nitric oxide, and leukotriene B_4 (Rodriguez-Cabezas et al., 2003).

Schley and Field, (2002) proposed at least three mechanisms that may explain the effects of total fibre on the GALT. First, fibre can serve as a substrate for the microflora in the intestine and enhance the growth of probiotics (e.g. lactobacilli and bifidobacteria), which may then positively affect the immune system by changing (increasing) the proportion of CD8 and CD4 positive T-cells (Schley and Field, 2002). Second, fermentation of fibre can increase the production of some SCFA, which may affect intestinal cells and the immune system. Third, mucin production may be altered affecting the immune system (Schley and Field, 2002). Therefore, fibre or its fermentation by-products may positively affect the GALT.

1.7. Effect of fibre on the GI tract

The GI tract may affect the general health of the person since the GI tract modulates the metabolic response to a meal (Schneeman, 2002). Dietary fibre may act directly on the GI tract by affecting its structure and functions (including barrier function) (Rehman et al., 2003) or may act indirectly on the luminal contents, which modulates the absorption and nutrient availability to the GI tract (Flourie et al., 1984).

Feeding diets containing 10% (w/w) fibre (cellulose or pectin) to rats for 4 weeks caused an increase in jejunal villus height and width compared to rats whose diet contained no fibre (Sigleo et al., 1984). Tasman-Jones et al., (1982) found that feeding rats a diet containing pectin (10% w/w) for 12 weeks caused the villi to differentiate and mature faster than a diet containing cellulose or one that was fibre-free. These findings are consistent with the increased intestinal length, villus height, and crypt depth seen in healthy rats fed diets containing soluble fibre (Andoh et al., 1999).

Several studies have suggested that dietary fibre may influence cell proliferation in the intestine. Gee et al., (1996) showed that rats fed guar gum had an increased crypt cell proliferation rate in the distal ileum. However, consuming wheat bran may reduce rectal cell proliferation (Alberts et al., 1990). Cameron et al., (2000) proposed that consuming a diet with 10% (w/w) fibre, fermentable in the colon, reduced cecal pH and reduced cell proliferation in the colon. The differences in cell proliferation may reflect the different area of the GI tract measured. The observed effects on cell proliferation may involve the generation of SCFA and / or hormonal mediator of intestinal cell growth.

Sakata (1995) proposed that the fermentation of fibre produces SCFA in the colon which may be crucial for maintaining the integrity of the small and large intestines. SCFA including butyrate, acetate, and proprionate (Klurfeld, 1999), may be used for energy by colonocytes (Roediger, 1995) and can stimulate a trophic effect in cells of the villi and crypts throughout the small intestine (Andoh et al., 1999). Friedel and Levine, (1992) examined the role of SCFA on colonic morphology and the absorptive capacities of the intestine for water and electrolytes in rats. Adult rats received nutrients via TPN formula that was either supplemented with SCFA or with saline (control). They showed that SCFA acted directly on epithelial cells of the GI tract to increase mucosal thickness of colon (Friedel and Levine, 1992). Andoh et al., (1999) and Goodlad et al., (1986) used similar protocols and showed that SCFA produced by fermentation of fibre lead to increased villus height and crypt depth.

Individual SCFA, for example butyrate, may have anti-inflammatory effects that help to reduce tissue damage in a rat model of colitis (Andoh et al., 1999). Kanauchi et al., (2001) found that the fermentable fibre of germinated barley foodstuff (GBF) caused an increase in butyrate production, which reduced mucosal damage and helped with tissue repair. Butyrate is used for energy by colonocytes (Roediger, 1995) and is a growth inhibitor (Beyer-Sehlmeyer et al., 2003) suggesting that butyrate and SCFA have numerous roles in the GI tract. Hence fermentable fibre serves as a substrate for the microflora, enhance their growth, activity, and production of SCFA (Blaut, 2002b;Charalampopoulos et al., 2002). These effects of fibre fermentation are associated with effects on the structure and function of the GI tract.

Intestinal hormones including glucagon-like peptide-2 (GLP-2), can also enhance the growth and structure of the small intestine (Burrin et al., 2001). Consumption of fibre can increase plasma levels of GLP-2 (Fukunaga et al., 2003) and GLP-2 messenger RNA (mRNA) in the ileum of rats (Reimer and McBurney, 1996). The increased gene expression correlated with an increase in plasma levels of the insulinotropic hormone, glucagon-like peptide-1 (GLP-1) (Reimer and McBurney, 1996). Feeding diets containing 26% w/w fibre (lignin, cellulose and hemicellulose) to diabetic rats increased plasma levels of GLP-2 compared to rats fed a fibre-free diet, but this difference was not seen in normal rats (Thulesen et al., 1999). Importantly, the trophic effect of GLP-2 on epithelial cells has been shown to enhance the intestinal barrier function after the administration of this hormone (Benjamin et al., 2000;Cameron et al., 2003b). Therefore, intestinal barrier function may be improved by GLP-2, which can be upregulated with the consumption of fibre.

Fibre can affect the function of the intestine by increasing fecal weight (Cummings and Macfarlane, 1993), improving laxation and hastening transit time through the GI tract (Lupton et al., 1993). Diets low in fibre were associated with constipation in children (Morais et al., 1999). Adding frutooligosaccharide (FOS), a fermentable fibre, to infant cereals led to more frequent and softer stools, without diarrhea, compared to infants fed regular infant cereals (Moore et al., 2003). Nonviscous fibre (ie. cellulose) was shown to reduce symptoms and severity of diverticular disease (Aldoori et al., 1998;Aldoori et al., 1994).

Intestinal barrier function is maintained or improved with the consumption of fibre. Gum Arabic, a soluble fibre, can help maintain tight junction structure and

paracellular transport in the face of substances such as chenodeoxycholic acid (CDC), protamine (PTM), and 2,4,6-triaminopyrimidine (TAP) that disrupt or block tight junctions (Rehman et al., 2003). The authors suggested that fibre enhanced the paracellular junctions or improved the structure of the intestinal epithelial cells which in turned lead to enhanced intestinal barrier function.

Deng et al., (1999) observed that the bacterial translocation was lower in rats fed chow or enteral formula with fiber (i.e. pectin) than those fed the fibre-free enteral formula. In addition, the lactulose: mannitol ratio was lower in rats fed the formula with fibre than those fed the enteral formula without fibre (Deng et al., 1999). Correa-Mattios et al., (2003) found that feeding FOS to piglets with an infection prevented the decrease in intestinal resistance seen in pigs fed the control fibre-free diets. Gyory and Chang, (1983) fed rats one of three diets; the control fibre-free diet or the control diet supplement with either 20% (w/w) bran or lignin. They measured cecal and colonic permeability using PEG markers and found that the bran diet was able to prevent the increase in permeability observed in the other two groups when exogenous bile acids were added. Therefore, these studies suggest that the intestinal barrier function was better in the groups fed fibre than those consuming diets without fibre.

The luminal content can be altered by fibre in the diet, which can affect various parameters in the intestines. For example, pectin added to a solution used to perfuse the jejunum increased the viscosity of the solution and increased the jejunal UWL, which reduced the amount of glucose absorbed (Flourie et al., 1984).

The type and viscosity of dietary fibre can affect the viscosity of the luminal contents influencing the GI tract and the production of SCFA. Elsenhans and Caspary

(2000) evaluated the adaptation of the intestine in rats fed diets containing different amounts of fibre with varying viscosities. In this study, female wistar rats were fed a fibre-free, control diet or the control diet supplemented with hydroxyethyl cellulose (HEC) of different viscosities (low, medium, and high) added at different concentrations (8,16, 32% w/w) for 6 weeks. At each concentration of HEC added to the diet, there was a low, medium, and high viscosity HEC group resulting in a total of nine groups. A cereal-based standard chow group was also included for comparison. Increasing the viscosity of the fibre caused a lengthening of the small intestine, regardless of the concentration of the fibre in the diet (Elsenhans and Caspary, 2000). Diets containing the higher viscosity HEC at the same w/w concentration of fibre resulted in heavier intestinal and mucosal weight compared to the diets containing low viscosity HEC.

In addition to the effects on structure, viscous fibre has been proposed to reduce the rate of absorption of glucose and some lipids (Battilana et al., 2001; Bourdon et al., 1999). Some researchers have postulated that the lower absorption rates may be due to an increase in the resistance of the UWL or the promotion of the formation of a gel-like substance in the lumen (Flourie et al., 1984). Therefore, viscosity of the fibre ingested can affect the viscosity of the luminal contents (Cameron-Smith et al., 1994;Elsenhans and Caspary, 2000), which may affect the absorption of some nutrients and intestinal structure.

1.8. β-glucan

1.8.1. Definition of β-glucan

β-Glucan is a cell wall polysaccharide found mainly in the endosperm of barley (70% of total β-glucan) and in the aleurone layer of oats (67% total β-glucan) (Cui, 2001). Small amounts of β-glucan are also found in rye (1-2%) and in wheat (<1%). The structure is similar among these grains and is composed of 2 to 3 (up to 14) glucose units linked by β (1→4) bonds which are separated by a β (1→3) linkage (Wood, 1990;Cui, 2001). The number of β (1→3) linkages may differ among the grains, which may change the solubility and perhaps the function of β-glucan (Wood, 1990;Cui, 2001). The length of the β-glucan chain is directly proportional to its viscosity in solution (Wood et al., 2000). β-Glucan is known to be a highly viscous fibre (Wood et al., 2003).

The total amount of β-glucan in a cereal varies with the plant's variety, the growing environment (Duffus and Coohrane, 1993), and post-harvest handling and processing (Kerckhoffs et al., 2003). β-Glucan is available naturally in whole grains such as barley and oats, but can also be extracted and purified. Extracting β-glucan from barley or oats may be a cost effective process to increase the amount of β-glucan in certain foods and in the diet (i.e. total dietary intake). Additional alkali/acids steps during the purification process may remove more protein, starch, and minerals. The resulting product contains a higher yield of the purified β-glucan than the product that has not undergone the additional purification steps. However the molecular weight (and viscosity) of the extracted β-glucan is diminished (Cui, 2001) which could reduce its functional properties (Kerckhoffs et al., 2003). Below is a summary of the procedures

used to extract high amounts of β-glucan without compromising the high viscous nature of the grain's β-glucan.

1.8.2. Overview of the Extraction Process for Candle barley-derived β-glucan.

For the thesis project, the waxy hull-less Candle barley flour was used to produce a high-purity β-glucan extract. Briefly, Candle barley flour was added to 2.2L of warmed (~54°C) distilled water (dH_2O) at a pH of 9.1 (adjusted with Na_2CO_3) and stirred for 1h to solubilize β-glucan and proteins. The remaining insoluble portion, which was enriched in starch, was centrifuged and the precipitate was discarded. The temperature of the supernatant was increased (85°C) to denature glucanase enzymes intrinsic in the barley grain to avoid β-glucan degradation. Maintaining the supernatant at high temperatures (85-90°C), lowering the pH to 6.3 and adding an amylase enzyme (Termamyl) digested the remaining starch. The solution was cooled (50°C) and pH lowered to 5 in order to denature the proteins and cause them to precipitate at their isoelectric point. The protein precipitate was separated by centrifugation and discarded, leaving the supernatant containing the β-glucan. The β-glucan was precipitated from the supernatant by adding an equal volume of absolute ethanol and the precipitate was recovered by filtration and washed with ethanol. β-Glucan was homogenized and dried under vacuum. The product was ground to provide a "powder-like flour" substance. The procedure produced a long chain, high-viscosity β-glucan product.

Low-viscosity β-glucan was prepared by subjecting the high-viscosity β-glucan solution to high shear in a microfluidizer. This procedure shortens the chain length of the

β-glucan molecule and produces the low-viscosity β-glucan extract. β-Glucan was recovered from the aqueous solution by ethanol precipitation (see Appendix 2).

1.8.3. Role of β-glucan in health and on the GI tract

β-Glucan has been shown to have health benefits similar to dietary fibre. Tappy et al., (1996) fed subjects with type 2 diabetes breakfast cereals containing varying amounts of β-glucan (4, 6 and 8.4g) or a continental breakfast. β-Glucan reduced the rise in postprandial glycemia, peak plasma glucose levels, and area under the curve for glucose. These effects were dose dependent, indicating that the effects were more pronounced at higher β-glucan concentrations compared to the lower concentration. The rise in insulin following the intake of the breakfast cereals containing β-glucan was about half that observed after the continental breakfast.

Bratten et al., (1994a) compared the effects of oat gum (a purified extracted form of β-glucan) and oat bran (a whole grain source of β-glucan) added to wheat farina porridge on postprandial glucose and insulin responses in people with or without diabetes. Intact β-glucan from natural sources (i.e. oat bran) and isolated β-glucan had similar effects in that they both attenuated the postprandial plasma glucose and insulin levels (Braaten 1994a). Therefore, incorporating β-glucan into breakfast cereals or other foods may be beneficial for those with diabetes (Braaten et al., 1994a;Franz et al., 2002;Tappy et al., 1996;Wood et al., 1994;Wood et al., 2000). These effects may result from a reduction in the glycemic index of foods containing β-glucan (Jenkins et al., 2002a;Vachon et al., 1988) and an associated delay in glucose absorption (Battilana et al.,

2001). The addition of one gram of β-glucan has been shown to reduce the glycemic index of foods by 4 units (Jenkins et al., 2002a).

Diets with β-glucan may affect serum lipid profiles. Jenkins et al., (2002b) evaluated the health claim in the USA about β-glucan and psyllium on lowering risk of CVD risks. For one month, hyperlipidemic people ate β-glucan and pysllium totaling 8g/d more soluble fibre compared to a control group (30g/d vs 20g/d total fibre, respectively). The total cholesterol concentration, the total cholesterol to HDL-C ratio, and the LDL-C: HDL-C ratio all decreased in the group that consumed the β-glucan and psyllium-containing diet.

Davidson et al., (1991) fed oatmeal or oat bran at doses of 1, 2, or 3 oz per day or a farina control diet to people for 6 weeks. Total and LDL-C levels were reduced after consuming at least 2oz / day of oat bran or oatmeal. At the same dose of oat bran 56 g (2oz) per day, there was a significantly greater LDL-C lowering effect than the diet with oatmeal. The authors suggested that the higher β-glucan content of oat bran most likely explained the greater effect on lowering LDL-C (Davidson et al., 1991).

Kerckhoff et al., 2003 carried out two studies in which subjects with hypercholesterolemia ate breads and cookies enriched with oat-derived β-glucan (study 1) or drank orange juice containing β-glucan or wheat (study 2). LDL-C concentrations were comparable between the treatment and control groups when the β-glucan was consumed in breads and cookies. However, LDL-C concentration were significantly lower by 0.26 ± 0.07 mmol/L after consuming orange juice with β-glucan than the juice with wheat fibre. This suggests that the cholesterol-lowering properties of oat-derived β-glucan are influence by food processing or mixtures.

β-glucan also affects the GI tract. A small quantity of the β-glucan is digested in the duodenum and jejunum leaving about 33-87% non digested (Knudsen, 1990;Sundberg et al., 1996). The β-glucan remaining may help increase the viscosity of the luminal contents in the upper small intestine (Knudsen, 1990). This viscosity may contribute to the attenuation of postprandial blood glucose concentrations (Vachon et al., 1988;Wursch and PiSunyer, 1997), possibly by reducing the intestinal absorption rate of glucose (Wood, 1990;Battilana et al., 2001). Increasing luminal viscosity may also contribute to lowering total cholesterol and LDL-C concentrations observed in some studies (Jenkins et al., 2000a;Jenkins et al., 2000b;Anderson et al., 2000).

β-glucan or its hydrolysates serves as an energy substrate for the microflora in the intestine. Jaskari et al., 1998 found that the hydrolysis products of oat β-glucan (e.g. β-glucooligomer) promoted the growth of the probiotics *Bifidobacterium sp.* and *Lactobacillus sp* in culture (Jaskari et al., 1998). Intestinal bacteria ferment the nondigested β-glucan and produced SCFA.

Dongowski et al., (2002) fed male Wistar rats one of four test diets that varied in the source and amount of β-glucan and resistant starch or fed a control diet containing no barley and very minimal β-glucan (0.03g/100g diet). After consuming the diet for 6 weeks, intestinal weight, the bacterial content, and the SCFA profile were assessed. Results showed that rats fed diets containing higher quantities of β-glucan (6.03-6.78g/100g diet) produced five times more SCFA than those fed the control diet (0.03g β-glucan /100g diet)(Dongowski et al., 2002). There was an increased total amount of SCFA in the cecum and colon of rats fed the diets with the highest amount of β-glucan (Dongowski et al., 2002; Dongowski et al., 2003). Fermentation of β-glucan in the colon

was positively associated with weights of the cecum and colon and the weight of their respective contents (Dongowski et al., 2002). There does not appear to be adverse side effects (e.g. diarrhea, growth restriction, water intake, red blood cell count, or clotting factor) in rats fed 7% (w/w) β-glucan for 28 days (Delany et al., 2003) suggesting that prolong consumption of β-glucan up to this level is safe.

β-Glucan in the diet affects other aspects in the GI tract and intestinal function. In a study by Lia et al., (1995), people with ileostomies ate, in random order, breads made with oats or barley that differed in the amounts of β-glucan, or a wheat control bread. The amount of bile acid excreted during a 24-hour period was higher in people after consuming the oat bran bread (12.5g β-glucan) compared to when they consumed breads made with barley (13g β-glucan), oat bran with β-glucanase (3.8g β-glucan) or wheat (1.8g β-glucan). The presence of β-glucan and its structure were important in affecting the amount of bile acids excreted (Lia et al., 1995).

Bile acid excretion under different dietary conditions has also been studied in rats. In rats, the amount of primary and secondary bile acid excreted (i.e. in the feces) increases with the amount of β-glucan in the diet (Dongowski et al., 2002). Dongowski et al., (2003) used a similar protocol to their previous study (Dongowski et al., 2002) and fed rats one of five diets containing varying amounts of β-glucan and resistant starch for 6 weeks. The amount of primary bile acids in the distal small intestine, cecum, and colon were highest in the rats fed 6.03g or 6.78g β-glucan /100g diet (i.e. groups with the highest amount of β-glucan) versus the diets with 3.84g or 4.04g β-glucan/100g diet. The increased bile acid excretion occurring in the GI tract was proposed to contribute to the decrease in cholesterol levels in rats (Dongowski et al., 2003); a similar mechanism may

be responsible for lowering cholesterol in humans with (Brighenti et al., 1999;Lia et al., 1995) and without (Braaten et al., 1994b;Mcintosh et al., 1991) normal lipid profiles.

1.9. BioBreeding diabetes prone rats as a model of leaky gut

Evaluating the effect of fibre on intestinal barrier function necessitates having a model with normal and abnormal barrier functions. There are two strains of BioBreeding (BB) rats: one that is prone to the development of autoimmune diabetes (BBdp) and one that is not (BBn). BBn rats appear to have a regular intestinal barrier function, while BBdp rats have increased permeability in the gastric and upper regions of the small intestine prior to the onset of diabetes (Meddings et al., 1999). These rats were first identified in the early 1970's when a subgroup of the BB colony developed polyuria, glycosuria, polyphagia, and weight loss that mimicked symptoms of type 1 diabetes in humans (Marliss et al., 1983;Nakhooda et al., 1976). Further studies have found that the BBdp rats have low numbers of CD4+ T-cells, relatively no CD8+ T-cells in their peripheral blood (Edouard et al., 1993) and that they lose tolerance to self antigens, which may lead to autoimmune diabetes (Mathieu et al., 1994). Therefore, the BBdp rats can be used as a model for increased intestinal permeability and altered barrier function, as well as for type 1 diabetes. The BBn rats serve as a control strain.

1.10. Summary

The GI tract is the first place where fibre interacts with the body, making the intestine an important organ to evaluate when assessing the effects of fibre on the body. A fundamental role of the intestine is to limit the passage of pathogens and potentially

harmful antigens from the environment to the rest of the body. This barrier function may be augmented by the intake of dietary fibre. In rats, studies that delivered nutrients via TPN formulae have found that fibre prevented or reduced the destruction of the intestinal barrier that is a consequence of a TPN feeding protocol (Deng et al., 1999). TPN diets containing FOS as a source of dietary fibre were shown to maintain the integrity of the barrier by preventing the reduction in epithelial resistance caused by an infection (Correa-Matos et al., 2003). Inclusion of dietary fibre increases villi height and crypt depth compared to a fibre-free diet (Andoh et al., 1999). Fibre also increases mucosal mass via increased cell proliferation or GLP-2 and this may help to maintain the epithelial barrier (Cameron et al., 2003a). Fibre and its fermentation by-products (i.e. SCFA) may enhance the health of the GI tract by reducing inflammation (Kanauchi et al., 2001) and reducing proinflammatory cytokines in the GALT (Rodriguez-Cabezas et al., 2003).

β-glucan is a cell wall polysaccharide found in oats, barley, rye, and wheat (Cui, 2001) and can be extracted from oats and barley. β-glucan in the diet of humans have been shown to reduce postprandial blood glucose and insulin levels in the acute situation (Battilana et al., 2001; Bourdon et al., 1999; Braaten et al., 1994a) and may reduce total cholesterol and LDL-C concentrations in the longer-term (Braaten et al., 1994b;Kerckhoffs et al., 2003). Other studies showed that β-glucan augments the excretion of bile acids in humans (Lia et al., 1995) and in rats (Dongowski et al., 2003), enhances the growth of probiotic bacteria, and increase the production of SCFA (Dongowski et al., 2002). These SCFA may also be associated with epithelial cell growth as observed with other fibres. However, the effects of purified β-glucan in the diet on the intestinal barrier have not been evaluated previously.

Chapter 2

Rationale

Consumption of nondigestible plant materials may have health promoting effects such as reducing total cholesterol and LDL-C concentrations (Anderson et al., 2000;Brown et al., 1999), attenuating the postprandial rise in blood glucose and insulin (Braaten et al., 1994a), hastening intestinal transit, and stimulating the growth of probiotics (Blaut, 2002). Furthermore, the morphology (villi height and crypt depth) (Andoh et al., 1999) and the unstirred water layer thickness (Flourie et al., 1984) of the small intestine increases in response to fibre in the diet. The number and structure of villi were enhanced when pectin was added to a standard diet (Tasman-Jones et al., 1982). Expression of intestinal hormones such as proglucagon mRNA may increase in the ileum of rats fed an elemental diet supplemented with fibre (Reimer and McBurney, 1996).

Several studies and review articles (Danielson et al., 1997;Jenkins et al., 2000b;Jenkins et al., 2002a;Malkki and Virtanen, 2001;Malkki, 2001;Wursch and PiSunyer, 1997) have suggested that the viscosity of dietary fibre is important in affecting health and GI functions. The viscosity of the fibre is proposed to relate to the viscosity of luminal contents (Cameron-Smith et al., 1994;Danielson et al., 1997; Elsenhans and Caspary et al., 2000). An increased viscosity of the fibre and luminal contents have been associated with a reduction in cholesterol levels (Vuksan et al., 2000), a reduction in the glycemic control marker, fructosamine (Vuksan et al., 1999), and a reduction in the risk of insulin resistance (Jenkins et al., 2000a;Vuksan et al., 2000).

β-Glucan is a plant cell wall polysaccharide found primarily in oats and barley, and to a lesser extent, in rye and wheat. Some varieties of barley can contain high levels of β-glucan and can be used as a source to extract β-glucan. β-Glucan from oats and barley has been shown to reduce total cholesterol and LDL-C concentrations (Braaten 1994b) and to attenuate postprandial blood glucose and insulin concentrations (Braaten et al., 1994a; Bourdon et al., 1999).

One of the food-related health claims in the USA suggests that β-glucan may be the active ingredient in oats that reduces plasma cholesterol concentrations (HHS Food and Drug Administration, 2001). Currently, products containing β-glucan derived from oats, but not from barley, can carry the health claim in the USA that associates a reduction in cholesterol and risk for cardiovascular disease (CVD) with the consumption of oats. The majority of the studies (Jenkins et al., 2002b; Lia et al., 1995; Kerckhoffs et al., 2003) used β-glucan from oat sources (e.g. oat bran, gum or concentrate). Subjects in a study by Bourdon et al., (1999) consumed pasta enriched with barley-derived β-glucan and had reductions in their cholesterol concentrations. Thus, the data suggests that β-glucan from either oats or barley may be beneficial. However, studies using barley-derived β-glucan are needed to establish barley and its derivatives as a beneficial crop capable of promoting health and carrying a health claim.

Functional aspects of the GI tract are also affected by β-glucan. For example, intake of β-glucan has been shown to cause an increased excretion of bile acids (Lia et al., 1995), an increased number of *Lactobacilli* bacteria in the intestine, and augment the production of SCFA (Dongowski et al., 2002). SCFA, which are by-products of fibre fermentation, may mediate the trophic effects of fibre on the small intestine (Klurfeld,

1999). Less is known, however, about the role of β-glucan and fibre on the intestinal barrier function.

The intestine has a critical role in maintaining a barrier to potential harmful bacteria and antigens while absorbing nutrients that the body needs (Söderholm, 1998) The GI tract encounters a plethora of challenges; the integrity of the barrier may prevent infections and help to maintain the health of the host. The barrier can be compromised by bacteria (Garcia-Lafuente et al., 1998), immunosuppressive drugs (Dias et al., 1998;Madsen et al., 1995a), and by fasting (Bragg et al., 1991). On the other hand, the intestinal barrier function can be enhanced by nutrients such as fibre. It is proposed that the UWL thickens in response to fibre (Flourie et al., 1984), which may slow the absorption of nutrients as well as pathogens. Adding fibre to enteral formulas has been shown to reduce bacterial translocation to the mesenteric lymph nodes (Mosenthal et al., 2002) and to reduce intestinal permeability (Correa-Matos et al., 2003). However, there is insufficient data on the effect of ingesting a diet with different types of fibre from barley (whole grains versus extracted β-glucan) on the intestinal barrier function. Since the GI tract is the first place where food and β-glucan first interacts with the body, evaluating the effects of β-glucan on intestinal barrier is critical in assessing its nutraceutical effects (see appendix 1 for overview of barley project).

The aim of this project was to evaluate the effects of the type of fibre (barley-derived β-glucan, barley flour, oatmeal or cellulose) in the diet on small intestinal permeability and morphology. In addition, the project aimed to assess whether the viscosity (high or low) of the β-glucan affected the permeability of the jejunum and ileum. Therefore, it was hypothesized that:

Hypothesis 1:

In BBn rats with normal gut function, the permeability, morphology and the number of IgA positive cells will differ between rats fed a diet contain a β-glucan extract compared to those fed the barley, oatmeal, or cellulose diets.

Hypothesis 2:

In BBn rats with normal gut function, the permeability, morphology and the number of IgA positive cells will differ between rats fed the high viscosity β-glucan (HVβG) vs the low viscosity β-glucan (LVβG) diet.

Hypothesis 3:

In BBdp rat with abnormal gut function, the permeability, morphology and the number of IgA positive cells will not differ between rats fed a diet contain a β-glucan extract compared to those fed the barley, oatmeal, or cellulose diets.

Hypothesis 4:

In BBdp rats with abnormal gut function, the permeability, morphology and the number of IgA positive cells will not differ between rats fed the HVβG vs the LVβG diet.

Chapter 3

Methods

3.1. Overview

The intestinal function / morphology studies evaluated the effect of different types (alkali extracted vs. whole grain) and viscosity (high or low) of β-glucan on intestinal parameters. Intestinal permeability, electrophysiological parameters, morphology, and the presence of IgA positive cells in intestinal sections were assessed. These studies were carried out using BioBreeding normal (BBn) and –diabetes-prone (BBdp) rats that were fed one of five diets for 14 days. The study reported in this thesis was part of a larger project outlined in appendix 1.

A second trial was conducted to determine the incidence of diabetes in the Agricultural, Food, and Nutritional Science department BBdp rat colony fed a semi purified, casein-based diet containing cellulose as the fibre source. Rats were fed the cellulose diet from weaning until 120 days of age or the onset of diabetes, which ever came first (see appendix 4).

3.2. Animals

BBn and BBdp rats were obtained from the colony in the Agricultural, Food, and Nutritional Science department at the University of Alberta. Animals were originally purchased from the Sir Frederick Banting Research Centre (Health Products and Food Branch, Health Canada, Ottawa ON, Canada). These studies were approved by the

Faculty of Agriculture, Forestry and Home Economics' Animal Policy and Welfare Committee (protocol 2002-19C) and the animals were treated according to the Canadian Council on Animal Care guidelines. Rats were housed in a temperature and humidity controlled rooms with a 12 hour light – dark cycle. For all studies, pregnant dams were fed a standard rat chow (5001 Rodent Diet: Lab Diet, Brentwood MO, USA) until their litters were born. On the day of birth, the dams' diet was switched to a semi-purified casein-based diet containing cellulose as the fibre source (ie. cellulose or control diet). Dams remained on this diet until the rat pups were weaned.

3.2.1. Intestinal function / morphology study

At 21 days of age, the rats were removed from their mothers and housed in groups of 1-4 pups / shoebox cage. All animals had access to food and water *ad libitum* throughout the study. At 35 ± 2 days of age, rats were sacrificed and organs were collected as described in section 3.4. The sample size for each diet group in the the BBn and BBdp studies, respectively, are listed as follows: HVβG (15, 11), LVβG (15,11), barley (15,12), oatmeal (15, 13), and cellulose (19, 17).

3.3. Diets

3.3.1. Gut function / morphology study

At weaning, rat pups were randomly assigned to receive 1 of 5 diets from 21 to 35 days of age (see Figure 1).

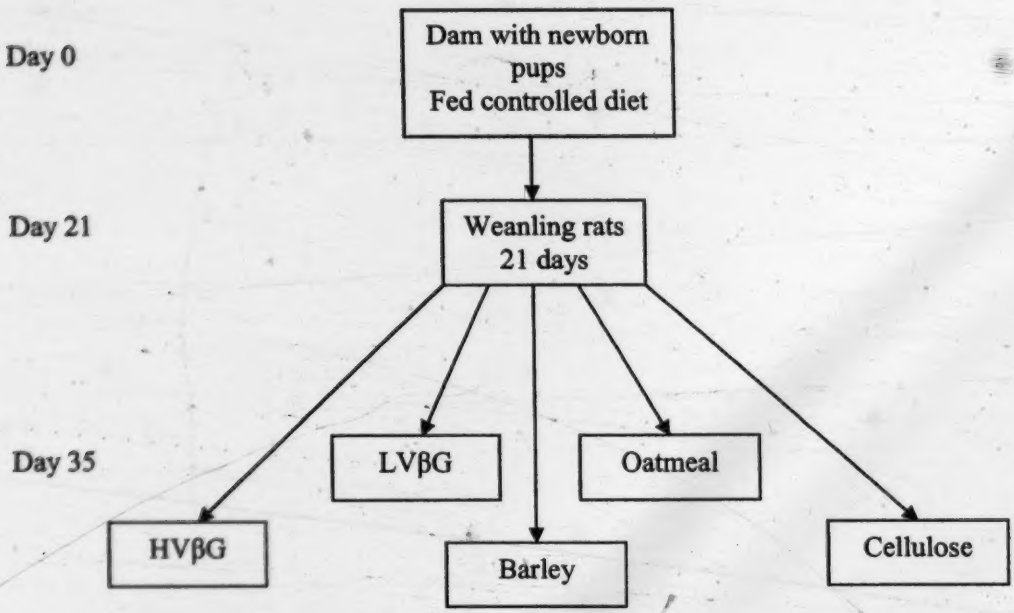

Figure 1. Design of gut function / morphology trial (BBn & BBdp).

The five diets were made in our laboratory. The diets were semi-purified, casein-based with only the fibre composition differing. All of the diets contained approximately 8% (w/w) total fibre, derived from a combination of one of four sources of fibre and non-nutritive cellulose. The sources of dietary fibre were: high purity high viscosity β-glucan (HVβG), high purity low viscosity β-glucan (LVβG), barley, oatmeal, and cellulose. The macronutrient and fibre composition of the diets are shown in Table 1. The nutrient composition (protein, starch, lipid, and β-glucan) for each batch of β-glucan and oatmeal was determined prior to its use in preparing the diets. The amount of each diet ingredient was adjusted to maintain a constant macronutrient and fibre profile for each diet.

Table 1 (a). Amount and source of macronutrients and fibre in the diets

	Diet				
Macronutrient	HVβG	LVβG	Barley	Oatmeal	Cellulose
Total amount in diet (g)	**1010.4**	**1015.1**	**997.0**	**989.2**	**999.9**
Total Protein (g/kg)	**267.8**	**269.8**	**264.8**	**266.9**	**270**
casein	263.3	266.7	258.3	259	270
β-glucan	4.5	3.1	-	-	-
barley flour (protein)	-	-	6.5	-	-
oatmeal flour (protein)	-	-	-	7.9	-
Total Lipids (g/kg)	**200.4**	**200.4**	**197.3**	**201.5**	**200.4**
soybean stearine	131	131	129	130	131
safflower	62	62	60	61	62
linseed	7.4	7.4	7.1	7.2	7.4
barley flour	-	-	1.2	-	-
oatmeal flour	-	-	-	3.3	-
Total Carbohydrates (g/kg)	**379.2**	**382.2**	**376.2**	**372**	**378**
corn starch	378	381.7	346.7	330	378
β-glucan	1.2	0.5	-	-	-
barley flour	-	-	29.5	-	-
oatmeal flour	-	-	-	42	-
Total Fibre (g/kg)	**78**	**78.8**	**79.2**	**77.3**	**80**
cellulose	22	22	75.3	75	80
β-glucan	56	56.8	-	-	-
barley flour (β-glucan)	-	-	3.9	-	-
oatmeal flour (β-glucan)	-	-	-	2.3	-

Table 1(b). Summary of the percentage of macronutrients and fibre in each diet

	Diet				
Nutrient (% w/w)	**HVβG**	**LVβG**	**Barley**	**Oatmeal**	**Cellulose**
protein	27	27	26.5	27.0	27
lipids	20	20	20	20	20
carbohydrates	37.6	37.7	37.7	37.7	37.8
fibre	7.7	7.8	7.9	7.8	8.0

Thus, all diets contained approximately 25%, 34%, and 41% of total energy derived from protein, carbohydrate, and fat, respectively. Micronutrients were added to

the diets in the form of a vitamin mix A.O.A.C (Association of Official Agricultural Chemist, 10g/kg diet) and the mineral mix, Bernhart-Tomarelli (50g/kg diet, Harlan Teklad, Madison WI, USA). The composition and amount of each ingredient in the vitamin mix and mineral mix are listed in table 2 and 3, respectively.

Table 2. Composition of vitamin mix and amount of each ingredient in 1kg formula

Ingredient	g/kg diet
Vitamin A and D Powder	0.04
Dry vitamin E acetate	0.2
Menadione (vitamin K3)	0.005
Choline dihydrogen citrate	4.88
P-aminobenzoic acid	0.1
Inositol	0.1
Niacin	0.04
D-calcium pantothenate	0.04
Riboflavin	0.008
Thiamine HCl	0.005
Pyridoxine HCl	0.005
Folic acid	0.002
Biotin	0.004
Vitamin B12	0.03
Dextrose, anhydrous	4.55

Table 3. Mineral mix composition and quantity of each ingredient in g/kg formula

Ingredient	g/kg diet
Calcium carbonate	21
Calcium Phosphate, dibasic	735
Magnesium oxide	25
Potassium sulfate	68
Sodium chloride	30.6
Sodium phosphate, dibasic	21.4
Cupric citrate	0.46
Ferric citrate, USP	5.58
Manganese citrate	8.35
Potassium iodide	0.0072
Zinc citrate	1.33
Citric acid	2.28

Source for both vitamin and mineral mixes: Harlan Teklad. Custom Research Diet catalog.

3.4. Organ Collection

At 35 ± 2 days of age, rats were anesthetized with halothane (MTC Parmaceutical, Cambridge ON, Canada) and 2-5 ml of blood collected by cardiac puncture (22G1" needle attached to a 5 ml syringe: Becton Dickinson, Franklin Lakes NJ, USA). Blood was divided between two vacutainers; one containing sodium heparin alone (Becton Dickinson, Franklin Lakes NJ, USA) and the other containing sodium heparin and aprotonin (10µl, 6 TIU: Sigma, St. Louis MO, USA). Vacutainers filled with blood were kept on ice until they were centrifuged (3000 rpm for 10 minutes, 4°C); the plasma was separated from the red blood cells, transferred to a microcentrifuge tube, and stored at -70°C until analyzed for various metabolites (lipid profile markers) by Dr. Tapan Basu's lab. Death of the rats was ensured by cervical dislocation.

Rats' abdomens were rinsed with ethanol (ETOH), and a midline incision was made to expose the intestine and internal organs. The spleen and mesenteric lymph nodes were removed under sterile conditions and placed in Krebs-Ringers-Hepes (KRH) buffer and kept on ice for isolation of lymphocytes by Dr. Catherine Field's lab. The small intestine was excised from the stomach to the ileocecal valve. Once excised, the intestine was held at one end and slightly extended on the lab bench along a measuring tape and the length was measured and recorded. The intestine was divided into three equal sections representing the duodenum, jejunum, and ileum. For BBn rats, the three intestinal sections were placed on a surgical board on the bench top for cleaning and segmenting. For BBdp rats, the three intestinal sections were place on a cooled surgical board to enhance the survival of the intestinal tissue.

From each section (duodenum, jejunum, and ileum), a 2-3 cm sample from the proximal end was cut, flushed with cold phosphate buffered saline (PBS) (pH 7.4), placed in a histological cassette, and put in modified Bock's solution (37% formaldehyde, 24%; glacial acetic acid, 5%; and distilled water, 71%) for 48 hours. The next 3 to 5 cm section was used for either gut-related hormones or permeability measurements depending on whether the segment was from the duodenum or jejunum and ileum. Permeability and electrophysiological parameters were assessed on the jejunum and ileum segments, only. Therefore, the 3 to 5 cm in the duodenum that followed the histological sample was collected and frozen in liquid nitrogen for determining gut-related hormones. In the jejunum and ileum, the 3-5 cm section adjacent to the histological sample was removed and processed for permeability and electrophysiological parameters. Each section was tied with a silk suture at one end, filled with PBS and clamped at the other end with a hemostat. The intestinal loop was placed in a 50 ml centrifuge tube filled with PBS and kept on ice until mounted in the Ussing chamber. The next 3-5 cm section of the duodenum and jejunum containing digesta was placed in a 20 ml scintillation vial and frozen in liquid nitrogen for the assessment of microflora by Dr. Gerald Tannock's laboratory. However, prior to the collection of the microbiology section (3 to 5 cm) in the ileum, a 3 cm piece was gathered for gut-related hormones assessment. The remaining pieces of the intestine were placed in a Petri dish containing sterile PBS and used to isolate Peyer's Patches (Figure 2).

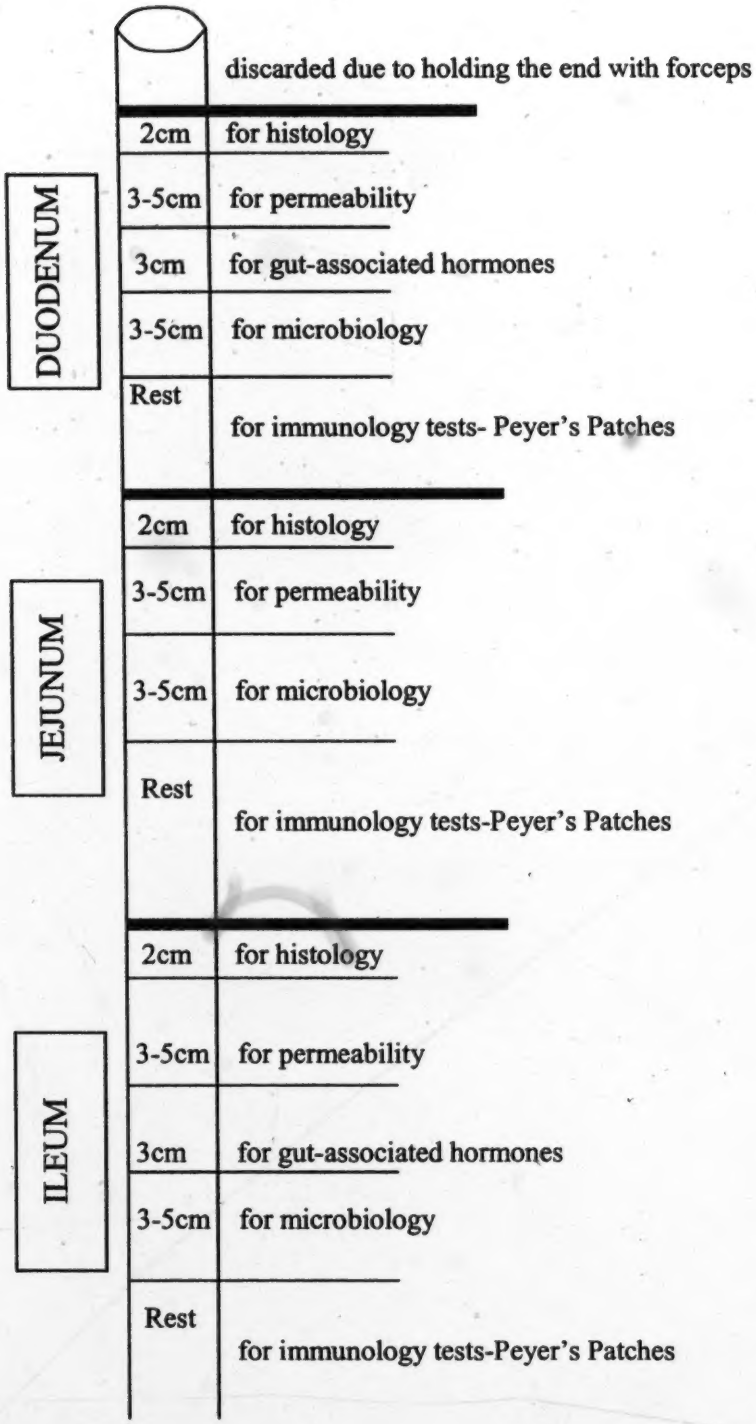

Figure 2. The segmentation of the small intestine for histology, permeability, hormone, and immunology measurements.

The cecum, stomach, and liver were excised, snap frozen in liquid nitrogen, and stored at –70°C. Samples collected for the assessment of proglucagon and intestinal hormones, immune system, and microflora will not be further discussed in this thesis.

3.5. Assessment of permeability and electrophysiological parameters

Jejunum and ileum segments, devoid of Peyer's Patches and stripped of their serosa, were cut along the mesenteric border and mounted in voltage clamped Ussing chambers. Each side of the tissue was bathed in 10 ml of bicarbonate Ringer's solution (1.1 mM $MgCl_2$, 1.25 mM $CaCl_2$, 114 mM NaCl, 5 mM KCl, 25 mM $NaHCO_3$, 1.65 mM Na_2HPO_4, 0.3 mM NaH_2PO_4) containing 1mM mannitol and 20 mM fructose (200ul of 1M fructose / chamber) that was circulated with 95% O_2 and 5% CO_2 and maintained at 37°C. Tissues were allowed to equilibrate for 15 minutes, during which ^{3}H-mannitol (10µl, 1mCi: PerkinElmer Life Sciences, Boston MA, USA) was added to the reservoir to which the mucosal side of the tissue was exposed (i.e. the mucosal reservoir). Two initial samples (100µl each) were withdrawn from the mucosal reservoir and added to a 5 ml scintillation vial containing 900µl of Ringer's solution. Samples (1000 µl each) were withdrawn from the other reservoir (ie. serosal reservoir) every 5 minutes during a 20-minute time period for calculating the flux of mannitol. Each 1000 µl was replaced by the same quantity of Ringer's solution containing 10 mM fructose. After the last sample was taken from the serosal reservoir, two samples (100µl each) were collected from the mucosal reservoir. Scintillation fluid (4 mL, Ready Safe) was added to each vial and

radioactivity in each sample was determined using a scintillation counter (5 minutes in beta ray scintillations).

During the two minutes prior to the collection of each flux sample, the tissue's electrophysiological parameters were recorded. Two percent agar AgCl current and calomel voltage electrodes were connected to the Ussing chamber and to an EVC3 preamplifier (World Precision Instrument, Sarasota Fl, USA). Short circuit current (I_{sc}) and spontaneous potential difference (PD) were produced and detected by the current and voltage electrodes, respectively. Electrophysiological parameters (I_{sc}, PD) were recorded from the EVC 4000 Precision V/I clamp, ie. voltage clamp (World Precision Instruments, Saratosa Fl, USA). Conductance (G) was calculated from I_{sc} and PD readings using Ohm's law. Forskolin (Sigma MO, USA), an adenyl cyclase activator which increase cyclic adenosine monophosphate (cAMP) levels, was added to the serosal reservoir after all of the flux samples were collected. I_{sc} and PD readings were recorded at the peak of changes in the short circuit current (ie. 3-4 minutes after the addition of forskolin). These electrophysiological parameters were used to verify the tissue's viability and ability to secrete ions in respond to a stimulus.

3.6. Embedding, sectioning, and staining of intestinal samples

Intestinal samples were fixed in modified Bock's solution for 48 hours, washed three times with PBS and stored overnight in PBS at 4°C. The following day, the tissues were washed three times with PBS (pH 7.4) and stored in 10% (w/v) phosphate buffered formalin (Fisher, Edmonton, Canada).

3.6.1 Tissue Embedding

Tissues in their cassettes were left under running tap water overnight and were then dehydrated by passing them through a series of graded ETOH and cleared with xylene (Fisher, Fairlane NJ, USA) (Table 4).

Table 4. Tissue embedding: dehydration steps.

Step	Solution	Duration
1	50% (v/v) ETOH	1 hour
2	70% (v/v) ETOH	1 hour
3	95% (v/v) ETOH	1 hour
4	100% (v/v) ETOH	0.5 hour
5	100% (v/v) ETOH	0.5 hour
6	Xylene	0.5 hour
7	Xylene	0.5 hour

Intestinal tissues were soaked in paraffin (Surgipath, Winnipeg MB, Canada) for 20-30 minutes to allow the paraffin to adhere to the tissue (i.e. saturate the tissue). The intestinal sections were then cut perpendicular to the intestinal ring, placed upright in plastic molds (model # 03040, Surgipath, Winnipeg MB, Canada), embedded in melted paraffin and place on ice.

3.6.2. Intestinal morphology

Each paraffin-embedded intestine was sectioned (3-5μm thick) and mounted on Histobond slides (Marienfield,Germany). One slide with the ileum section from each rat was stained with hematoxylin and eosin (H&E) for measurement of villus height and crypt depth. Slides were evaluated using a light microscope (Leitz Dialux, Wetzlar, Germany) at a magnification of 125x (10x objective x 12.5x eye piece). Metamorph Meta Imaging Series V.5.0r7 (Univeral Imaging Corporation) was used to capture the

pictures from each sample and to measure villus height and crypt depth. Villus height is expressed as the average height of 2-10 intact well-oriented villi. A line was drawn from the tip to the base of the villus. Crypt depth is expressed as the average length of six to twelve intact crypts.

3.6.3. Immunohistochemistry

Pieces of the ileum from rats in the gut function / morphology studies were sectioned (3-5µm) onto slides. Slides were warmed in an oven (60°C) for 10 minutes, allowing the paraffin to melt and the tissue to adhere to the slide. The slides were deparaffinized, cleared, and hydrated through a series of clearing and hydration steps (Table 5). Endogenous peroxidase activity was blocked by soaking the slides sections in 0.3% hydrogen peroxide / methanol solution for 15 minutes. Slides were rinsed under tap water, placed in citrate buffer (1M citric acid, pH 6.5), and warmed at 90°C for 30mins. The slides were cooled in a 4°C fridge for 15 minutes and washed three times with PBS. Tissue sections were then covered with 20% goat serum (2ml in 8ml PBS) (Gibco, Burlington ON, Canada) for 20 minutes to prevent non-specific binding. The goat serum was discarded and the sections were covered with mouse anti-rat monoclonal IgA antibody (Sigma, St. Louis MO, USA) (1:200 dilution in PBS). Sections were incubated with the primary antibody (Ab) overnight in a moist chamber at 4°C. After 16 to 18 hours of incubation, the primary Ab was washed away with PBS and sections were incubated with the secondary Ab, bovine anti-goat IgG Ab (Vector Laboratories, Burlingame CA, USA) (1:200) for 20 minutes at room temperature. Slides were washed three times with PBS for a total of 5 to 6 minutes. The avidin: biotinylated enzyme complex (ABC)

(Vector Laboratories, Burlingame CA, USA) was added to each tissue section and the slides were incubated for 40 minutes. The slides were washed three times with PBS and then incubated with diaminobenzine tetrahydrochloride (DAB) (BioGenex, San Ramon CA, USA) for 5 minutes or until the tissue section turned a light brown colour. Slides were counterstained with Harris's hematoxylin (Sigma, St. Louis, Missouri, USA). Slides were dehydrated by a series of graded ETOH, cleared twice with xylene, and cover slips were mounted using Permount (Fisher, Fair Lawn NJ, USA).

Table 5. Immunohistochemisty

Sequence	Procedure	Solution	Duration	Purpose
1	Heat slides in oven (60C)	None	10 minutes	Helps melt paraffin
2	Add slide to xylene	Xylene	5 minutes	Remove paraffin
3	Add slide to xylene	Xylene	5 minutes	Remove paraffin
4	hydrate	100% (v/v) ETOH	2 minutes	hydrate
5	hydrate	100% (v/v) ETOH	2 minutes	hydrate
6	hydrate	95% (v/v) ETOH	1 minutes	hydrate
7	hydrate	70% (v/v)	1 minutes	hydrate
8	Add slides to distilled water (dH_2O)	dH_2O	5 minutes	Hydrate tissue
9	Place slides in H_2O_2/methanol	0.3% (v/v) H_2O_2/methol	15 minutes	Block endogenous peroxidase
10	Wash	Running tap water	3 minutes	Wash
11	Heat slides at 90°C	Citrate buffer (pH 6.5 at 90°C)	30 minutes	Antigen retrival
12	Place in fridge	Same solution as #10	15 minutes	Cool down slides
13	Wash	PBS (pH 7.4)	5 minutes	Wash
14	1.Draw circle with Papen and 2. add blocking buffer	20% goat serum in PBS (50µl in 15ml)	15 minutes	1. Provide boundary to prevent leakage of Ab and 2. minimize

				background staining
15	Discard blocking buffer			
16	Add primary Ab	IgA: 10µl in 2 ml (1:200)	Overnight at 4°C	Detect presence of Ag
17	Remove primary Ab and wash	Wash: PBS	5 minutes	wash
18	Add secondary Ab	Anti-mouse IgG 5µl/ml PBS	20 minutes	Binds to 1°Ab & serve as substrate for enzyme reaction to produce colour
19	Wash slides	PBS	5 minutes	wash
20	Add ABC	ABC	40 minutes	Enzyme substrate
21	Remove ABC and wash slides	Wash:PBS	5 minutes	wash
22	Add substrate	Diaminobenzidine solution and H_2O_2	5 minutes	Locate peroxidase in tissue
23	Place slides in coupling jar filled with dH_2O	dH_2O	6-10 minutes	Rinse the DAB
24	Counterstain	Hematoxylin	1 minute	Blocks background staining
25	Add PBS	PBS to cover section	1 minute	
26	Wash	dH_2O	5 minutes	rinse off residual hematoxylin
27	Dehydrate	70% (v/v) ETOH	1 minute	Dehydrate
28	Dehydrate	95% (v/v) ETOH	1 minute	Dehydrate
29	Dehydrate	100% (v/v) ETOH	1 minute	Dehydrate
30	Dehydrate	100% (v/v) ETOH	1 minute	Dehydrate
31	Clear	Xylene	5 minutes	Clear
32	Clear	Xylene	5 minutes	Clear

Eight villi per sample were counted. The variability expressed as 95% confidence interval for the number of IgA+ cells ranged from the least variability (4.1 to 4.6) to the highest variability (4 to 5.6) ranges.

3.7. Statistical analysis

Data for BBn and BBdp rats were separated and analysed by strain because the studies using BBn or BBdp rats were conducted at different times. All of the body weight data were analysed using a two-way analysis of variance (ANOVA) with diet and gender as independent variables (IV). Since rats were, on average housed two per cage and randomly assigned, female and male rats were housed together. Therefore, gender was combined for the one-way ANOVA analysis of food intake. Gut length, gut length per body, spleen weight, and spleen weight per body weight were normally distributed in BBn and BBdp rats, resulting in a two-way ANOVA conducted using diet and gender as IV. Comparison of diet effects on mannitol flux across the intestinal tissue were assessed with a one-way ANOVA. Electrophysiological parameters were assessed using a one way ANOVA with diet as IV. When the overall ANOVA test was significant, Tukey's post hoc analysis was conducted to determine differences among the diet groups. Since simple but not complex comparisons were conducted, no additional restrictions were needed on the p-value for the post hoc analysis. Pearson's correlation was used to assess the association between the amount of β-glucan and permeability measurements. Chi square analysis was used to compare the incidence rate between the two dietary groups in the BBdp rats. Statistical significance was achieved when the $p<0.05$.

Chapter 4

Results

4.1. Characteristics of BioBreeding normal (BBn) rats

Gender had a significant ($p<0.05$) effect on body weight, weight gain, and gut length per body weight. Therefore, data for these outcome measurements are presented by gender and diet. Data for permeability measures were combined for male and female rats because it did not differ between them.

4.1.1. Body Weight

Initial body weights were similar among diet groups ($p=.795$) and did not differ significantly ($p=.286$) between male and female rats. Since female and male rats grew differently (ie. gender had significant effect on growth, $p=0.001$), data for female and male rats are presented separately.

In female rats, final body weight of those fed the HVβG diet were lighter at the end of the study than those fed the oatmeal diet ($p=0.002$), but were of similar weight to the LVβG ($p=0.747$), barley ($p=0.084$) or cellulose ($p=0.269$)-fed rats. There were no other statistically significant differences among diet groups for final body weight ($p>0.098$) (Figure 3).

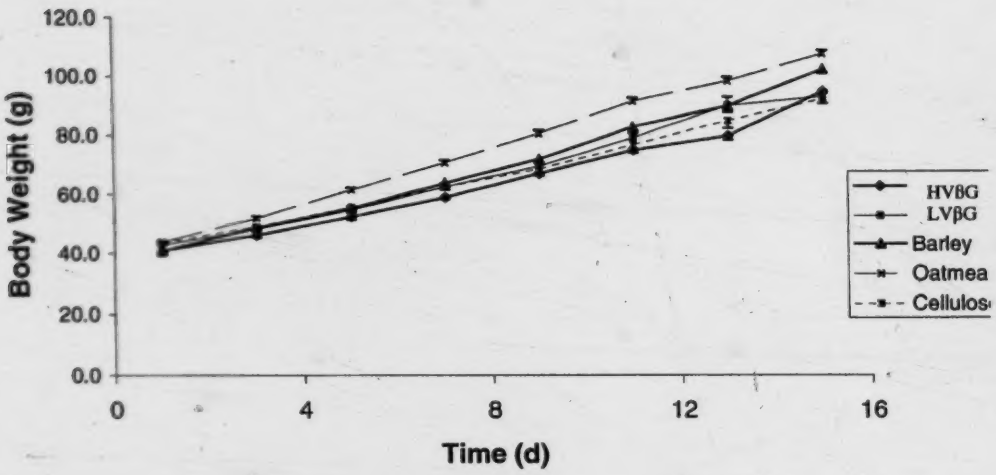

Figure 3. Body weight of female BBn rats. Data are presented as means ± SEM

Female rats gained about 54.5 ± 1.72g during the 14 day feeding period, which was about 8g less than male rats (p=0.002). Diet also affected total weight gain (p=0.004). Rats fed the HVβG or LVβG diets gained a similar amount of weight during the 14 days feeding trial (p=0.653). The HVβG group, gained about 11g and 17g less than the barley (p=0.026) and oatmeal (p=0.001) groups, respectively. Weight gain was comparable between the barley and oatmeal groups (p=0.921) (Table 6).

After 14 days of consuming the test diets, male rats fed the oatmeal diet weighed more than the HVβG-fed rats (p=0.002). There were no other statistically significant differences for final body weight among the diet groups (p≥0.063) (Figure 4).

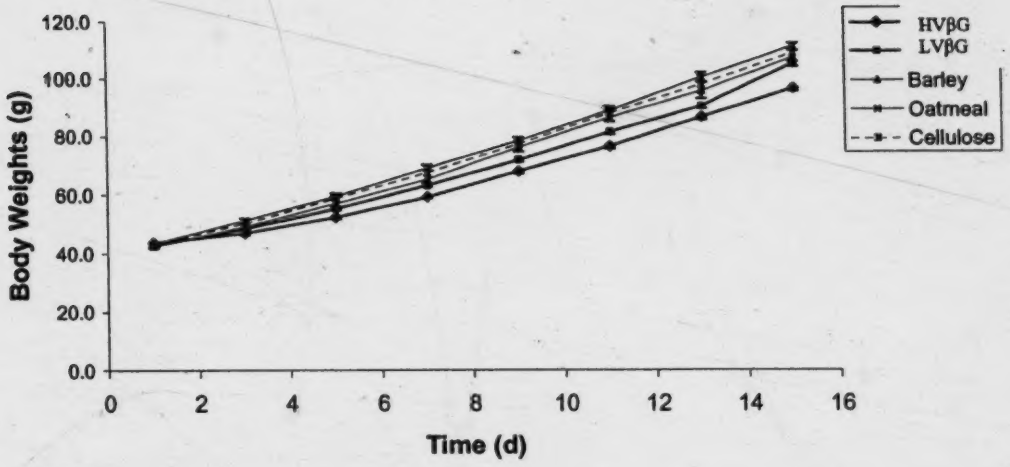

Figure 4. Body weights of male BBn rats. Data are presented as means ± SEM.

Male rats fed the HVβG diet gained less weight during the 14 day trial than the oatmeal ($p<0.001$) or barley ($p=0.026$) groups, but gained a similar amount of weight as the rats in the LVβG ($p=0.653$) or cellulose ($p=0.254$) groups (Table 6). There were no other statistically significant differences in weight gained among diet groups ($p\geq0.872$).

Table 6. Final body weight and total amount of weight gained during 14 days on the diets in female and male BBn rats.[1,2]

Diet	Final Body Weight (g)		Weight Gain (g)	
	female	male	female	male
HVβG	93.4 ± 2.8 (7)^{a}	97.3 ± 3.2 (13)^{a}	52.1 ± 3.0 (7)^{a}	53.4 ± 3.5 (13)^{a}
LVβG	93.0 ± 3.0 (6)^{ab}	105.1 ± 3.9 (9)^{ab}	50.3 ± 2.6 (6)^{ab}	62.4 ± 3.5 (9)^{ab}
Barley	102.2 ± 3.3 (6)^{ab}	107.1 ± 3.9 (9)^{ab}	61.5 ± 3.1 (5)^{b}	64.5 ± 3.8 (9)^{b}
Oatmeal	107.5 ± 3.3 (5)^{b}	111.1 ± 3.7 (10)^{b}	63.8 ± 3.1 (5)^{b}	67.7 ± 3.3 (10)^{b}
Cellulose	92.8 ± 2.6 (8)^{ab}	109.8 ± 3.4 (11)^{ab}	49.7 ± 3.7 (8)^{ab}	66.5 ± 2.2 (11)^{ab}

1. Data are presented as mean ± SEM (n)
2. Variables with different superscripts within a column are significantly different from each other, $p<0.05$

4.1.2. Food Intake

The amount of food consumed during the 14 day period differed according to the diet provided ($p<0.001$). Rats fed the diet containing HVβG ate less than those on the barley ($p<0.001$), oatmeal ($p<0.001$) or cellulose ($p=0.007$) diets. Food intakes of rats fed the LVβG were similar with the HVβG ($p=0.361$), barley ($p=0.247$), oatmeal (0.115) or cellulose ($p=0.618$) groups (Table 7). Barley and oatmeal-fed rats ate similar amounts of food ($p=0.995$).

Table 7. Total food intake for the 14-day feeding trial in BBn rats[1,2]

Diet	Food Intake (g)
HVβG	117.1 ± 4.3 (15)[a]
LVβG	129.7± 5.0 (15)[ab]
Barley	144.8 ± 5.0 (15)[b]
Oatmeal	147.5 ± 5.0 (15)[b]
Cellulose	139.5 ± 4.4 (12)[b]

1. Data are presented as mean ± SEM (n)
2. Variables with different superscripts are significantly different from each other, $p<0.05$

4.1.3. Gut Length

Diet ($p<0.001$) had a main effect on the length of the small intestine (Table 8). The small intestine was longer in rats fed the HVβG diet relative to those fed the barley ($p=0.003$) or cellulose ($p<0.001$) diets, but the intestinal length did not differ from rats fed the LVβG ($p=0.775$) or oatmeal ($p=0.131$) diets. Rats fed the diet containing LVβG had a significantly longer intestine than those fed the cellulose diet ($p=0.028$), but was similar in length to those fed the barley ($p=0.121$) or oatmeal ($p=0.784$) diets. On average, the small intestine of male BBn rats was 2 cm longer than that of female rats.

Expressing intestinal length per gram of body weight shows that gender (p=0.026) and diet (p=0.008) have an effect. Female rats have longer intestines when expressed per body weight than male rats fed the same diet. The LVβG dietary group had longer intestines than those fed the oatmeal (p=0.029) or cellulose (p=0.008) diets. Intestinal length expressed per gram body weight of rats fed the HVβG diet tended to be longer than the cellulose-fed (p=0.053) rats.

Table 8. Small intestinal length and length expressed per gram body weight in BBn rats[1,2]

Diet	gut length (cm)		gut length/body weight (cm/g)	
	female	male	female	male
HVβG	104.3 ± 1.7 (6)^{a}	104.6 ± 1.3 (10)^{a}	1.04 ± 0.04 (6)^{ab}	0.93 ± 0.03 (10)^{ab}
LVβG	99.3 ± 2.4 (3)^{ac}	103.9 ± 1.5 (8)^{ac}	1.04 ± 0.05 (3)^{a}	1.0 ± 0.03 (8)^{a}
Barley	97.3 ± 2.1 (4)^{bc}	99.0 ± 1.6 (7)^{bc}	0.91 ± 0.05 (3)^{ab}	0.90 ± 0.03 (7)^{ab}
Oatmeal	101.8 ± 2.1 (4)^{ab}	100.0 ± 1.6 (7)^{ab}	0.93 ± 0.04 (4)^{b}	0.86 ± 0.03 (7)^{b}
Cellulose	94.0 ± 1.9 (5)^{b}	99.5 ± 1.3 (10)^{b}	0.93 ± 0.04 (5)^{b}	0.85 ± 0.03 (10)^{b}

1. Data presented as mean ± SEM (n)
2. Variables within a column with different letters are significantly different at p<0.05

4.1.4. Spleen Weight

The spleen weight was affected by the diet (p=0.003) and gender in BBn rats. Specifically, spleens from rats fed diets containing HVβG or LVβG were not different from each other (p=0.789), but spleens from HVβG weighed less than the barley (p=0.039), or oatmeal (p=0.003) groups. There were no other differences (p>0.112) among the dietary groups (Table 9).

Table 9. Spleen weights of BBn rats fed one of six diets for 14 days[1,2]

Diet	spleen weight (g)	
	female	male
HVβG	0.46 ± 0.04 (7)[a]	0.48 ± 0.03 (12)[a]
LVβG	0.49 ± 0.04 (6)[ab]	0.53 ± 0.04 (9)[ab]
Barley	0.53 ± 0.04 (6)[b]	0.62 ± 0.04 (8)[b]
Oatmeal	0.63 ± 0.05 (5)[b]	0.60 ± 0.03 (10)[b]
Cellulose	0.46 ± 0.04 (8)[ab]	0.57 ± 0.03 (11)[ab]

1. Data are presented as mean ± SEM (n)
2. Variables with different superscripts within a column are different at $p<0.05$

4.2. Permeability of the Jejunum and Ileum

4.2.1. Mannitol Flux

Figure 5 shows the rate of mannitol flux in the jejunum and ileum segments of rats fed one of the five diets. In the jejunum, mannitol flux was not altered by diet ($p=0.959$) and ranged from 12.3 to 13.5nm/cm^2/hr. In the ileum, mannitol flux was significantly different among diet groups ($p=0.003$). Specifically, rats fed the LVβG diet had significantly higher mannitol flux compared to those fed the barley ($p=0.024$), oatmeal ($p=0.020$), or cellulose ($p=0.003$) diets, but it was similar to those fed HVβG ($p=0.458$) diet. Overall, there was an approximately 1.5 to 2 fold higher mannitol flux in the ileum of rats fed the LVβG diet versus the barley, oatmeal, and cellulose groups.

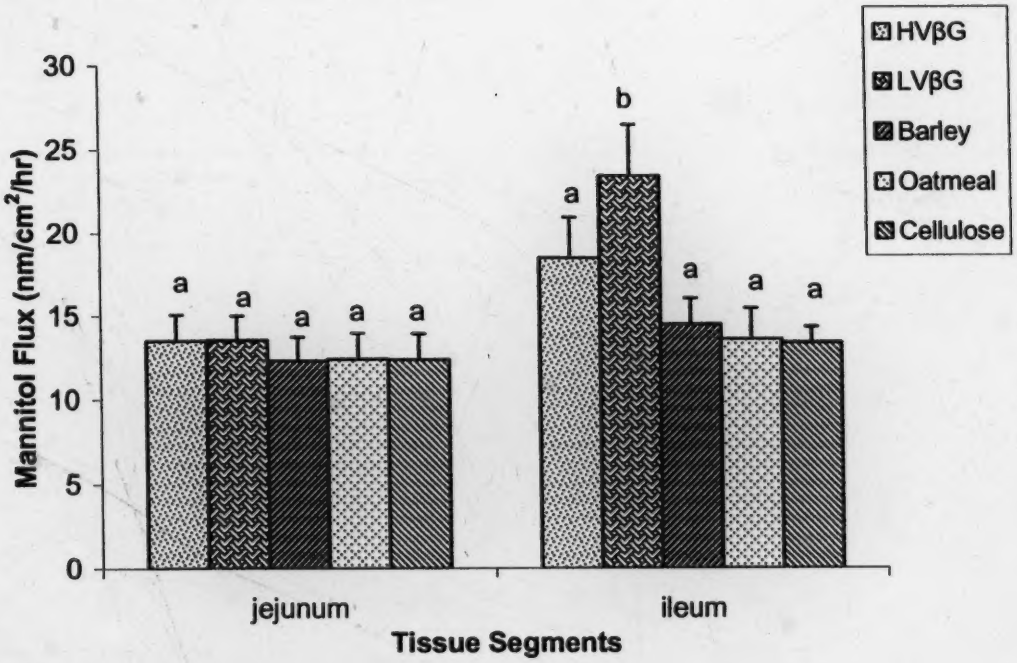

Figure 5. Mannitol flux in the jejunum and ileum of BBn rats.
Data are presented as mean ± SEM
Jejunum: HVβG (n=8), LVβG (n=10), barley (n=9), oatmeal (n=7), cellulose (n=7)
Ileum: HVβG (n=7), LVβG (n=9), barley (n=9), oatmeal (n=7), cellulose (n=10)

4.2.2. Electrophysiological Parameters

Conductance (G) represents the ability of tissue to regulate the overall movement of ions across the tissue through the transcellular and paracellular route. In other words, a high G value suggests that an increased number of ions are able to cross the tissue (Figure 6). In the jejunum, G was similar among the diet groups (p=0.775). There were no statistically significant differences among diet groups in the ileum (p=0.501).

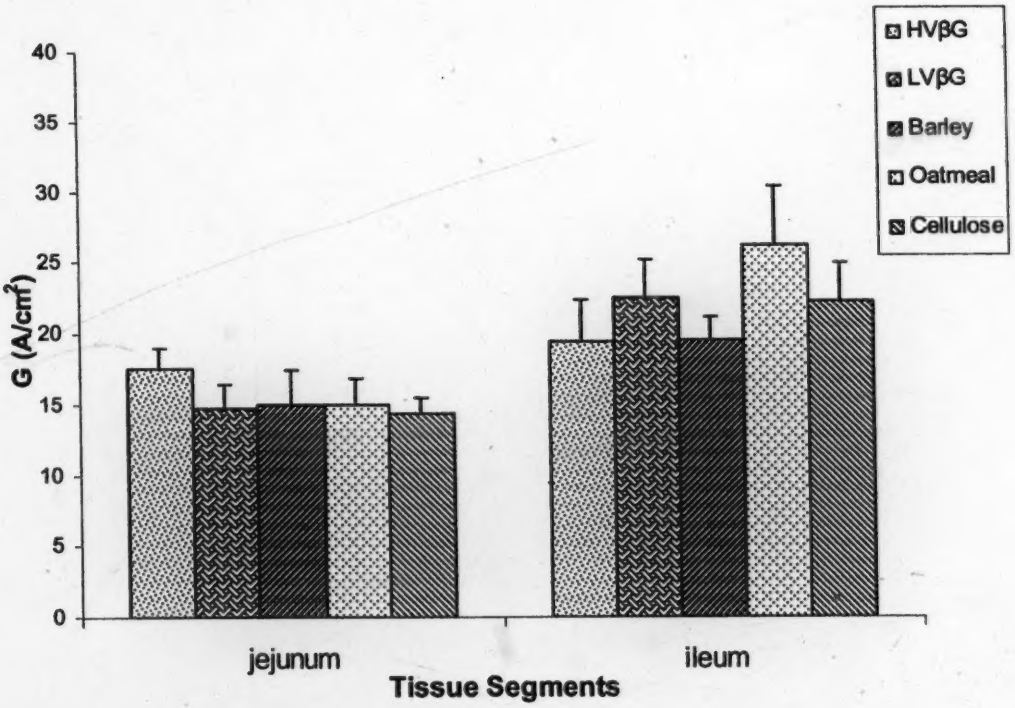

Figure 6. Conductance in the jejunum and ileum of BBn rats.
Data are presented as mean ± SEM
Jejunum: HVβG (n=8), LVβG (n=10), barley (n=9), oatmeal (n=7), cellulose (n=7)
Ileum: HVβG (n=7), LVβG (n=9), barley (n=9), oatmeal (n=7), cellulose (n=10)

Short circuit current (I_{sc}) reflects total bidirectional ion transport across the tissue. I_{sc} did not differ significantly among the diet groups in either the jejunum (p=0.358) or the ileum (p=0.417) (Figure 7). The jejunum and ileum of cellulose-fed rats had the lowest I_{sc} values relative to the other dietary groups, but the difference did not reach statistical significance.

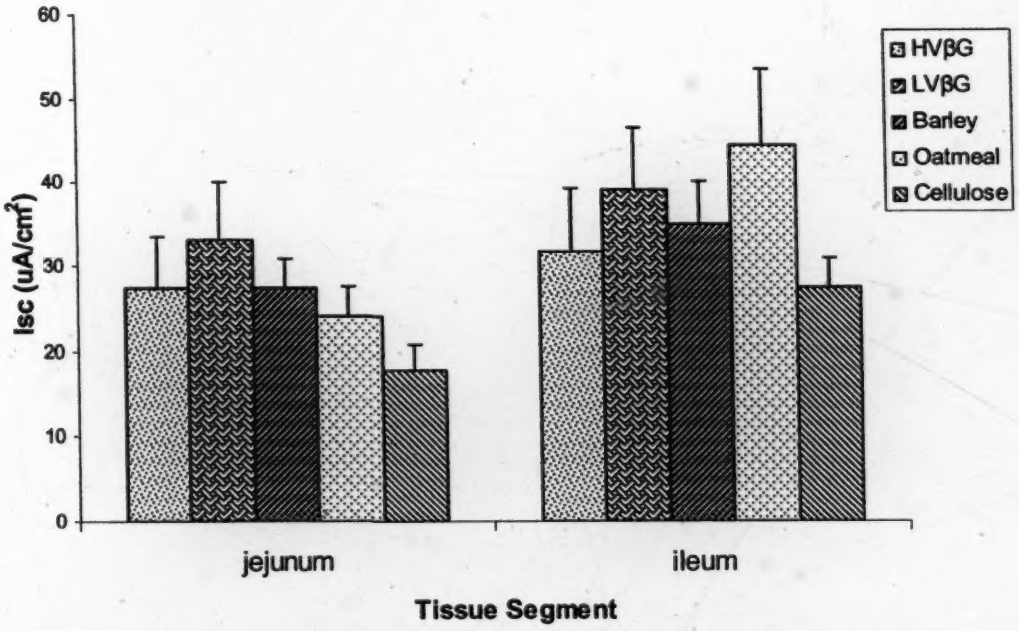

Figure 7. Short Circuit Current in the jejunum and ileum of BBn rats.
Data are presented as mean ± SEM
Jejunum: HVβG (n=8), LVβG (n=10), barley (n=9), oatmeal (n=7), cellulose (n=7)
Ileum: HVβG (n=7), LVβG (n=9), barley (n=9), oatmeal (n=7), cellulose (n=10)

Potential difference (PD) represents the ability of the tissue to maintain the ion (sodium/potassium/chloride) concentration gradients across the tissue, and to a lesser degree, the activity of the Na/K ATPase pump. It is primarily driven by sodium transport. PD readings in the jejunum and ileum did not differ significantly among diet groups ($p=0.125$, $p=0.642$, respectively) (Figure 8). In the jejunum, rats fed the LVβG diet had the highest PD. Rats that ate the diet containing cellulose had the lowest PD (as well as the lowest I_{sc}) in both the jejunum and ileum, yet these differences were not statistically significant ($p>0.05$).

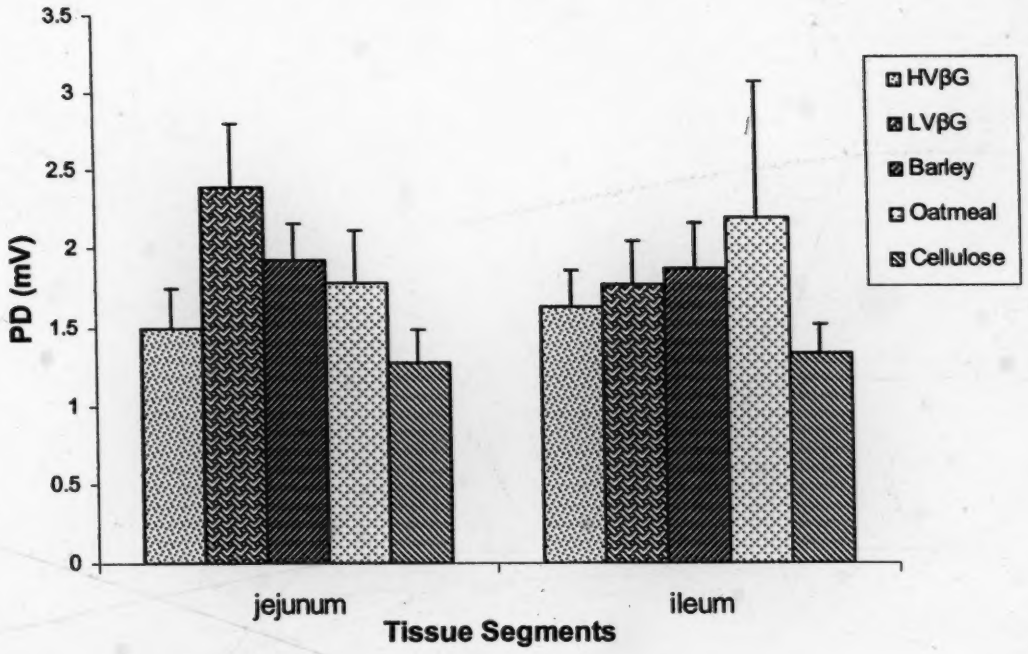

Figure 8. Potential Difference in the jejunum and ileum of BBn rats.
Data are presented as mean ± SEM
Jejunum: HVβG (n=8), LVβG (n=10), barley (n=9), oatmeal (n=7), cellulose (n=7)
Ileum: HVβG (n=7), LVβG (n=9), barley (n=9), oatmeal (n=7), cellulose (n=10)

Forskolin activates the adenyl cyclase enzyme and increases intracellular cAMP concentrations. This measurement is used to assess tissue viability at the end of the experiment and indirectly reflects ion, especially chloride, secretion. The change in I_{sc} in response to forskolin was similar among all diet groups in the jejunum ($p=0.467$) and in the ileum ($p=0.252$) (Figure 9). The jejunum from LVβG-fed rats had the highest forskolin response among the diet groups. The response to forskolin was the lowest in the HVβG-fed group, but the difference was not statistical significant ($p>0.05$).

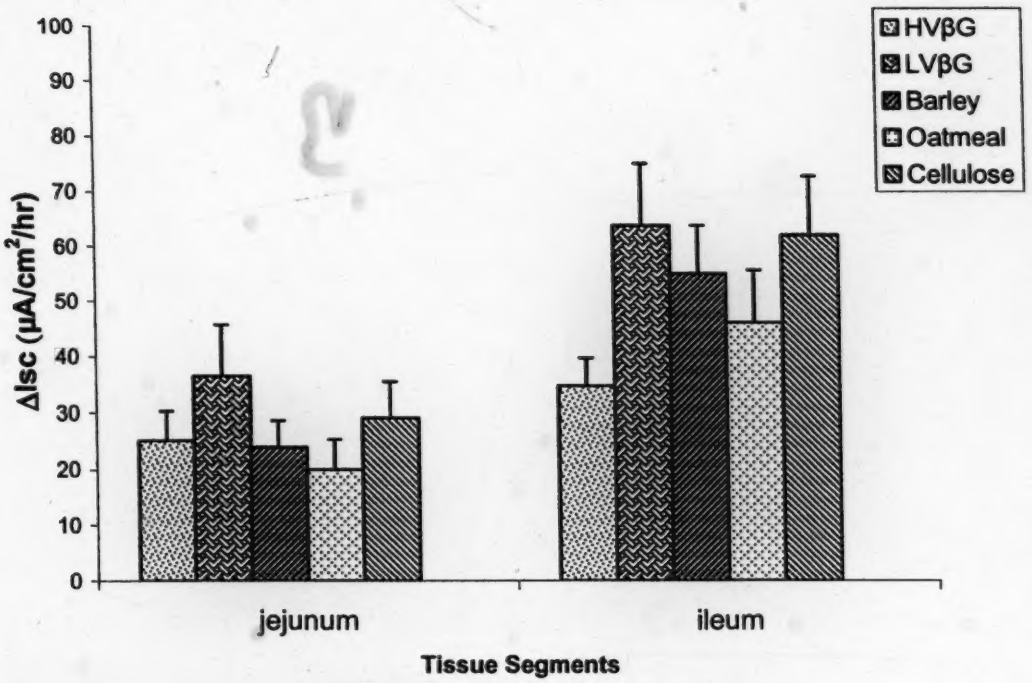

Figure 9. Response to forskolin in the jejunum and ileum of BBn rats.
Data are presented as mean ± SEM
Jejunum: HVβG (n=8), LVβG (n=10), barley (n=9), oatmeal (n=7), cellulose (n=7)
Ileum: HVβG (n=7), LVβG (n=9), barley (n=9), oatmeal (n=7), cellulose (n=10)

4.2.3. Correlation between the amount of β-glucan and permeability.

The difference among the test diets was the total amount of β-glucan. The amounts of β-glucan per kg diet were as follows: cellulose contained 0 grams, oatmeal (2.3g), barley (3.8g), HVβG and LVβG (56g each).

When the ileum was analysed separately from the jejunum, there was a significant and positive association ($r=0.515$, $p<0.001$) between the amount of β-glucan and mannitol flux in the ileum (Figure 10), but not in the jejunum. Therefore, as the amount of β-glucan increased, the mannitol flux increased. The difference was most apparent with the

largest amount (56g) of β-glucan in the diet. There were no significant correlations between the other electrophysiological parameters and the amount of β-glucan in the diet ($p \geq 0.300$).

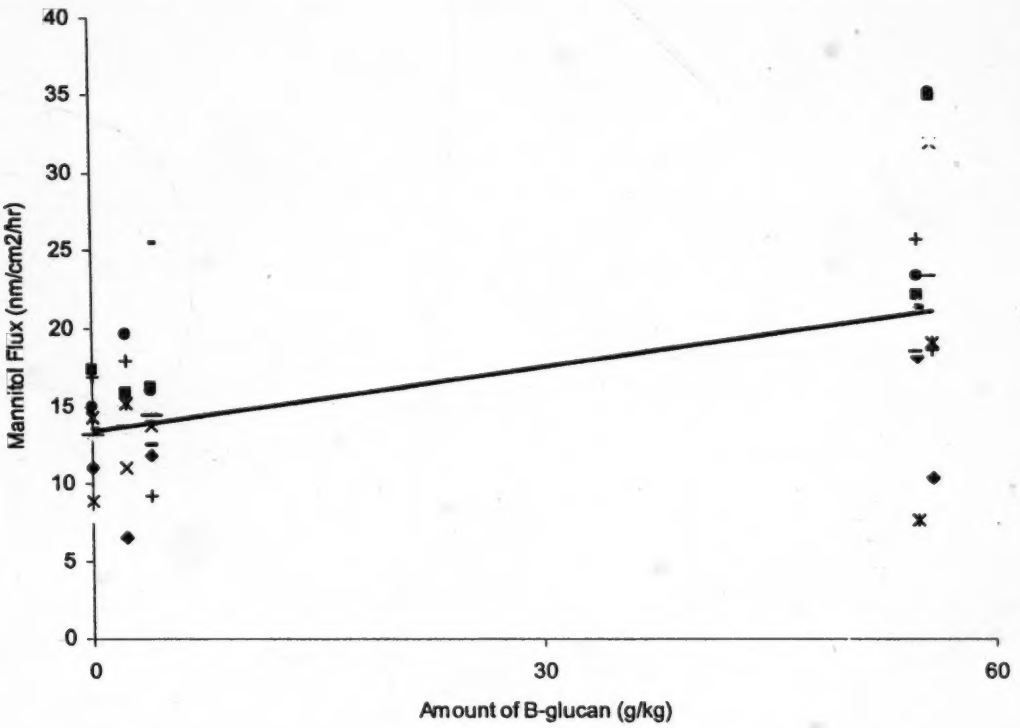

Figure 10. Correlation between the amount of β-glucan in the diet and mannitol flux in the ileum of BBn rats.

4.3. Morphology of the ileum

Diet had no effect on villus height ($p=0.359$). The villus height ranged from 424 to 467 μm, with the LVβG having the lowest average (Table 10). However, no differences were observed among these diets.

The depth of the crypts were not affected by diet (p=0.263). Rats fed the LVβG diet had the lowest average crypt depth at 132 μm. Oatmeal had the longest crypts at 143 μm, yet none of these differences were significant (Table 10).

Table 10. Villus height and crypt depth in the ileum of BBn rats.[1]

Diet	Villus Height (μm)	Crypt Depth (μm)
HVβG	467 ± 16 (10)	138 ± 2.39 (10)
LVβG	424 ± 14 (8)	132 ± 3.10 (8)
Barley	442 ± 15 (9)	137 ± 3.08 (10)
Oatmeal	464 ± 16 (11)	143 ± 3.13 (11)
Cellulose	440 ± 24 (7)	139 ± 4.97 (9)

1. Data presented as mean ± SEM (n)

4.4. Immunohistochemistry staining for IgA

An increased number of IgA positive cells in the intestine indicates that the immune system has responded to an exposure to antigens. The number of IgA positive per villi did not differ among diet groups (p=0.426) (Table 11). These results suggest that diet did not increase exposure to antigens.

Table 11. Number of IgA positive cells in the lamina propria of the ileum.[1]

Diet	Number of IgA+ cells / villi
HVβG	4.1 ± 0.30 (8)
LVβG	4.4 ± 0.19 (9)
Barley	4.1 ± 0.30 (8)
Oatmeal	4.2 ± 0.31(9)
Cellulose	4.8 ± 0.35 (8)

1. Data presented as mean ± SEM (n)

4.5. BBdp rats

4.5.1. Body weight

Gender had a significant effect on final body weight, weight gain, intestinal length and intestinal length per gram body weight ($p>0.05$). Therefore, these data are presented separately by gender.

Initial body weights were similar among the diet groups ($p=.762$), although male rats weighed approximately 7.8g more than their female littermates ($p<0.001$). In female and male rats, diet had no main effect on final body weights ($p=0.122$) (figure 11 and 12, respectively). The difference between those groups with the highest and lowest weights was approximately 12g for female and 22g for male rats. The total amount of weight gained from 21 to 35 days of age was affected by gender ($p=0.002$) but not by diet ($p=0.069$) (Table 12). Male rats generally gained 17g more than female rats.

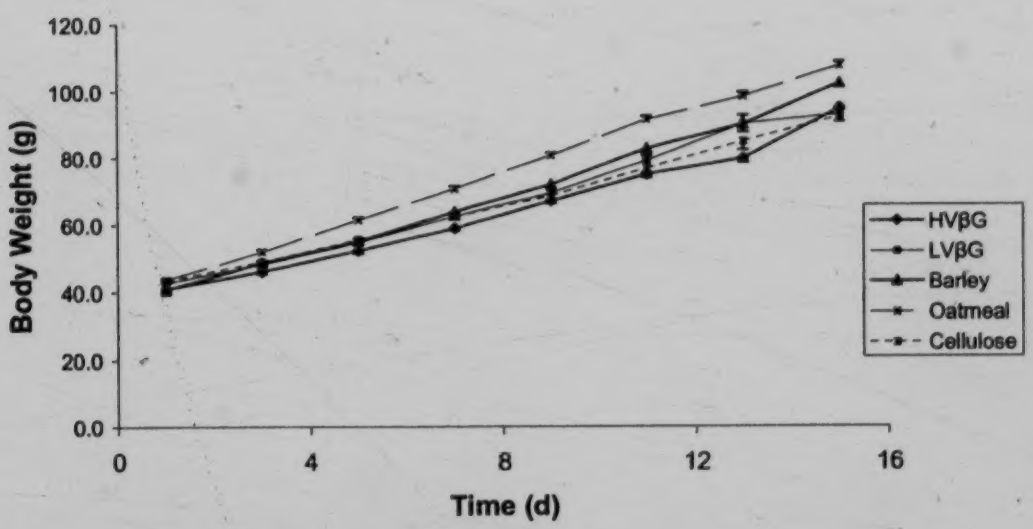

Figure 11. Body weight of female BBdp rats. Data are presented as mean ± SEM

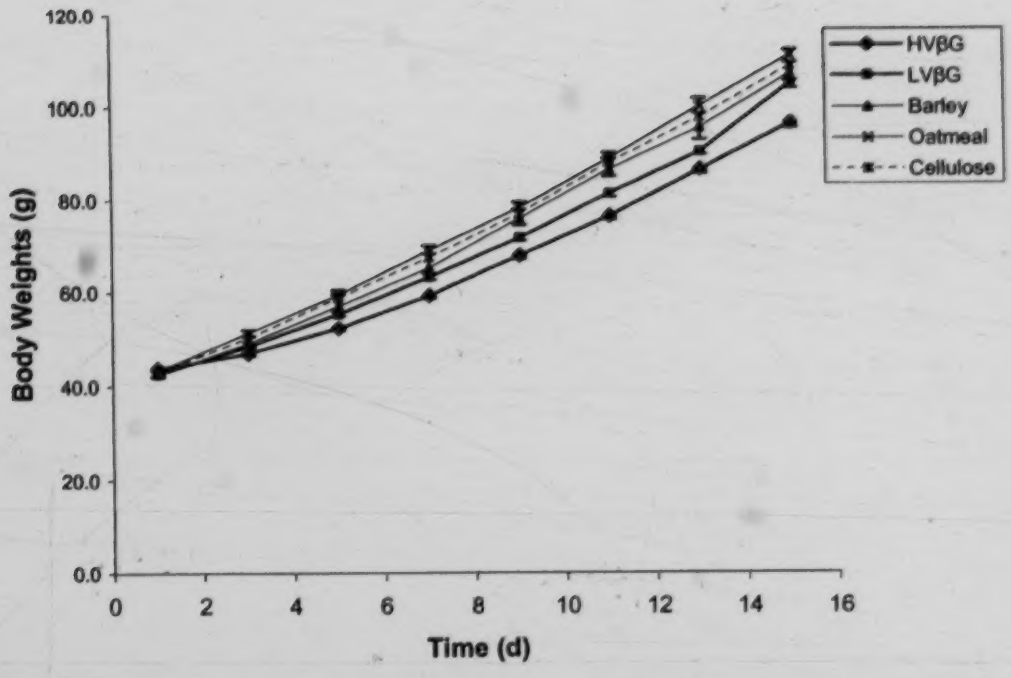

Figure 12. Body weights of male BBdp rats. Data are presented as mean ± SEM.

Table 12. Weights and total weight gain of female and male BBdp rats.[1,2]

Diet	Final Body Weight (g)		Weight Gain (g)	
	female	male	female	male
HVβG	89.3 ± 5.5 (7)	112.0 ± 8.3 (4)	46.2 ± 4.2 (7)	60.2 ± 5.6 (4)
LVβG	94.5 ± 8.5 (3)	115.3 ± 5.8 (8)	52.6 ± 6.4 (3)	62.1 ± 3.9 (8)
Barley	111.5 ± 7.3 (4)	124.2 ± 5.8 (8)	63.3 ± 5.6 (4)	70.3 ± 3.9 (8)
Oatmeal	101.6 ± 5.5 (7)	119.4 ± 6.7 (6)	59.2 ± 4.2 (7)	66.5 ± 4.5 (6)
Cellulose	100.0 ± 6.0 (6)	114.0 ± 5.2 (10)	51.6 ± 4.5 (6)	63.9 ± 3.5 (10)

1. Data are presented as mean ± SEM (n)
2. Gender affected final body wieght ($p<0.001$) and weight gain ($p=0.002$)

4.5.2. Food Intake

Diet had a significant effect ($p<0.001$) on the quantity of food that the rats consumed. Rats ate less of the HVβG diets compared with those fed the diet containing cellulose ($p<0.001$) or barley ($p=0.012$), but had similar food intake as those in the LVβG

group (p=0.419). Food intake was lower in rats that consumed the LVβG diet compared to the cellulose group (p=0.044). The rats in the HVβG group ate between 23 and 32g (over the entire study) less than those in the barley or cellulose (p≤0.012) groups (Table 13).

Table 13. Total food intake during the 14-day feeding trial for BBdp rats.[1,2]

Diet	Food Intake (g)
HVβG	113.2 ± 5.8 (8) [a]
LVβG	125.9 ± 5.8 (8) [ac]
Barley	137.1 ± 5.6 (9) [bc]
Oatmeal	129.9 ± 5.8 (8) [ab]
Cellulose	145.1 ± 4.8 (10) [b]

1. Data are presented as mean ± SEM (n)
2. Variables with different superscripts are significantly different from each other, p<0.05

4.5.3. Gut length and gut length per Body Weight

Gender (p=0.001), but not diet (p=0.074) had a significant effects on gut length in BBdp rats (Table 14). Female rats had intestines that were approximately 6 cm shorter than male rats fed the same diet (p<0.001).

Gut length is affected by overall body size. Therefore, data were analysed to assess the effects of diet on gut length relative to body weight. Diet had no effect on intestinal length per body weight (p=0.123), yet female rats had longer gut length than the males (p=0.006) (Table 14).

Table 14. Small intestinal length and length expressed per gram body weight in BBdp rats.[1,2]

Diet	gut length (cm)		gut length/body weight (cm/g)	
	female	male	female	male
HVβG	88.4 ± 2.3 (7)	99.4 ± 3.3 (4)	1.0 ± 0.05 (7)	0.90 ± 0.06 (4)
LVβG	90.7 ± 3.5 (3)	96.6 ± 2.1 (8)	0.98 ± 0.07 (3)	0.85 ± 0.04 (8)
Barley	94.8 ± 3.0 (4)	94.4 ± 2.1 (8)	0.88 ± 0.06 (4)	0.78 ± 0.04 (8)
Oatmeal	88.4 ± 2.3 (7)	97.7 ± 2.5 (6)	0.88 ± 0.05 (7)	0.83 ± 0.05 (6)
Cellulose	86.7 ± 2.5 (6)	90.3 ± 1.9 (10)	0.89 ± 0.05 (6)	0.80 ± 0.04 (10)

1. Data are presented as mean ± SEM (n)
2. Gender affected gut length (p=0.001) and gut length per body weight (p=0.006)

4.5.4. Spleen Weight

Spleen weight differed significantly between genders ($p<0.001$) but was not affected by diet ($p=0.204$) (Table 15). Female BBdp rats had smaller spleens than male rats ($p<0.001$).. Even after expressing spleen weight per gram body weight, diet had no effect on the size of the spleen ($p=0.779$).

Table 15. Spleen weights of BBdp rats fed one of six diets for 14 days.

Diet	spleen weight (g)	
	female	male
HVβG	0.28 ± 0.03 (7)	0.39 ± 0.04 (4)
LVβG	0.32 ± 0.04 (3)	0.41 ± 0.03 (8)
Barley	0.40 ± 0.04 (4)	0.38 ± 0.03 (8)
Oatmeal	0.33 ± 0.03 (7)	0.42 ± 0.03 (6)
Cellulose	0.30 ± 0.03 (6)	0.37 ± 0.02 (10)

1. Data are presented as mean ± SEM (n)
2. Gender affected spleen weight ($p<0.001$)

4.6. Permeability of the Jejunum and Ileum

4.6.1. Mannitol Flux

Mannitol flux did significantly differ among diet groups in the ileum ($p=0.047$) but not the jejunum ($p=0.375$) (Figure 13). In the ileum, the mannitol flux tended to be higher in rats fed the HVβG diet compared to those fed the cellulose diet ($p=0.066$). In the jejunum, rats fed the HVβG or LVβG diet had lower mannitol flux than the barley, oatmeal or cellulose group, but the difference did not reach significance ($p>0.369$).

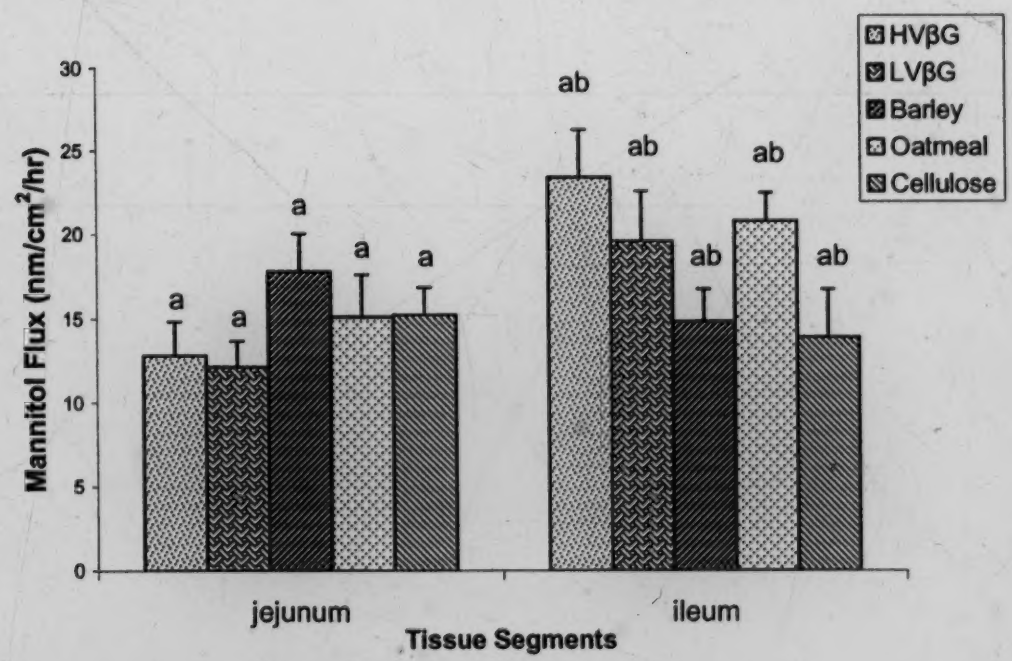

Figure 13. Mannitol flux in the jejunum and ileum of BBdp rats.
Data are presented as mean ± SEM
Jejunum: HVβG (n=10), LVβG (n=9), barley (n=11), oatmeal (n=12), cellulose (n=15)
Ileum: HVβG (n=11), LVβG (n=10), barley (n=12), oatmeal (n=9), cellulose (n=12)

4.6.2. Electrophysiological readings

There were no significant differences in G among the diet groups in the jejunum ($p=0.775$) or ileum ($p=0.171$). In the jejunum, G was lowest in HVβG fed groups. In the ileum, oatmeal fed rats had the highest conductance and G was similar amongst the other groups. However, the differences among diet groups in both tissue segments did not reach significance (Figure 14).

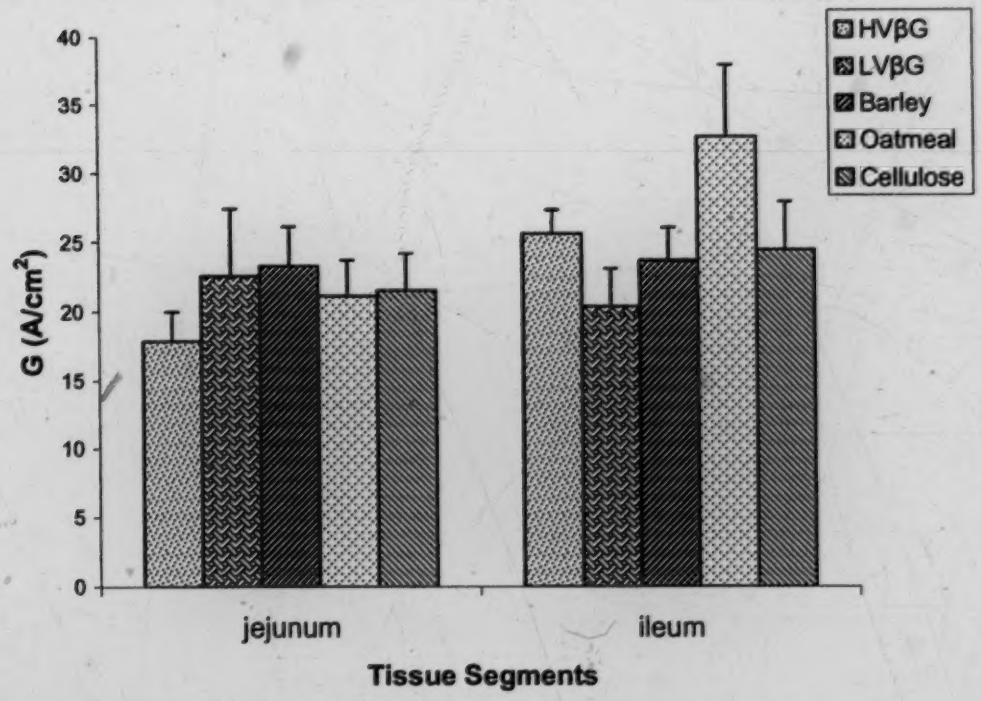

Figure 14. Conductance in the jejunum and ileum of BBdp rats.
Data are presented as mean ± SEM. Sample sizes are presented as diet (jejunum, ileum).
Jejunum: HVβG (n=10), LVβG (n=9), barley (n=10), oatmeal (n=12), cellulose (n=12)
Ileum: HVβG (n=10), LVβG (n=8), barley (n=11), oatmeal (n=10), cellulose (n=11)

I_{sc} readings were similar across the diet groups in both the jejunum (p=0.632) and ileum (p=0.852) tissue segments (Figure 15). The I_{sc} in the jejunum of rats fed the HVβG diet was the lowest of all the groups, but not statistically different (p>0.05).

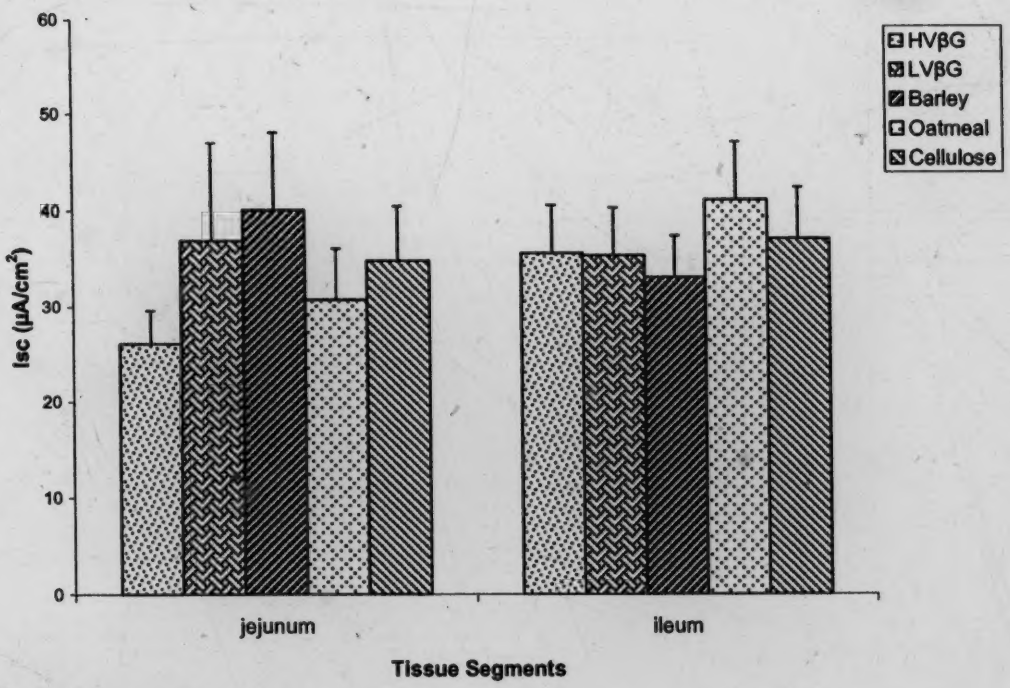

Figure 15. Short circuit current in the jejunum and ileum of BBdp rats.
Data are presented as mean ± SEM
Jejunum: HVβG (n=10), LVβG (n=9), barley (n=10), oatmeal (n=12), cellulose (n=12)
Ileum: HVβG (n=10), LVβG (n=8), barley (n=11), oatmeal (n=10), cellulose (n=11)

PD was comparable among the diet groups in the jejunum (p=0.875) and ileum (p=0.816) (Figure 16). PD in the jejunum of rats fed the HVβG diet was the lowest and barley-fed rats had the highest PD. LVβG-fed rats had the highest PD in the ileum. However, the differences were not significant (p=0.875, p=0.816, respectively).

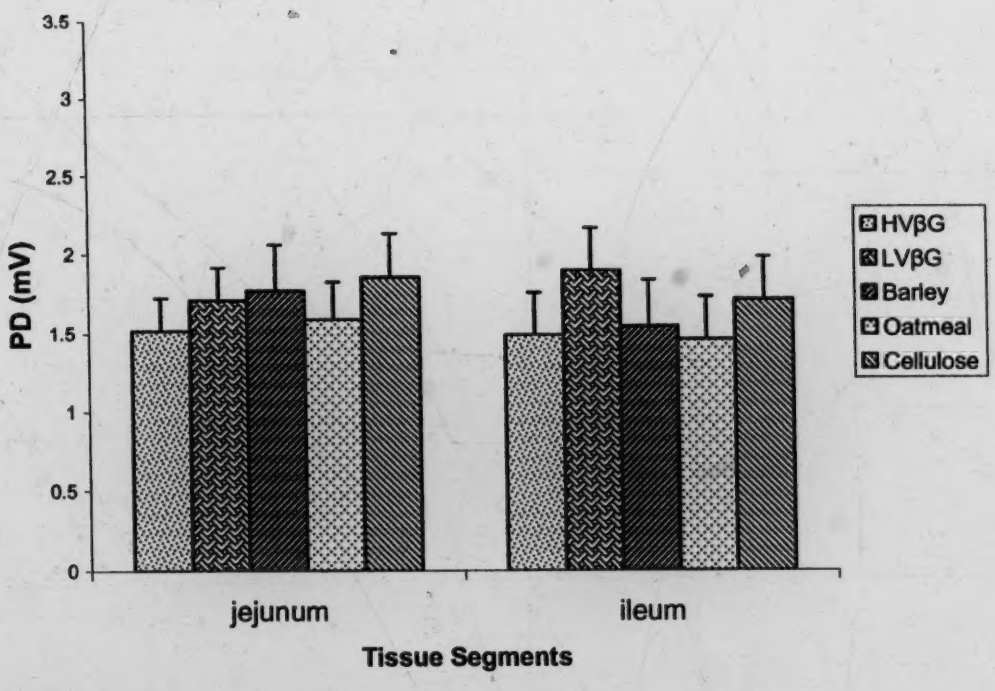

Figure 16. Potential Difference in the jejunum and ileum of BBdp rats.
Data are presented as mean ± SEM
Jejunum: HVβG (n=10), LVβG (n=9), barley (n=10), oatmeal (n=12), cellulose (n=12)
Ileum: HVβG (n=10), LVβG (n=8), barley (n=11), oatmeal (n=10), cellulose (n=11)

Adding forskolin to the jejunum produced a similar response among the diet groups (p=0.199). However, in the ileum, the responses were affected by diet (p=0.020). In the ileum, the oatmeal group had a significantly higher forskolin response than the barley group (p=0.022) and tended to be higher than the cellulose diet group (p=0.050). Although, the absolute response in the ileum of the oatmeal group was higher than those fed the HVβG and LVβG diets, it did not reach statistical significance (p=0.081, p=0.088, respectively) (Figure 17).

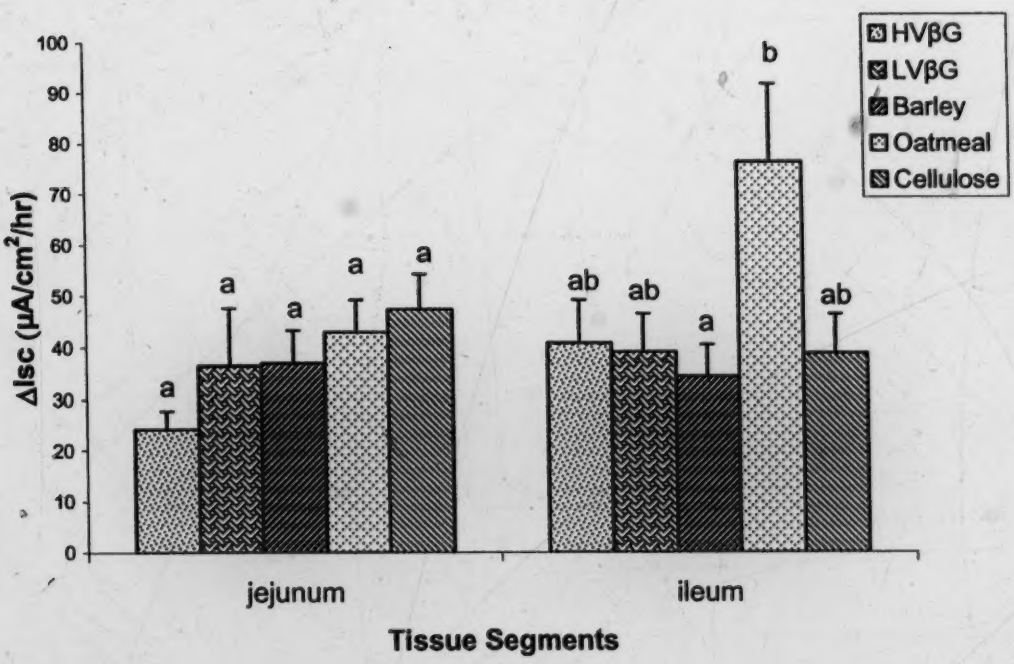

Figure 17. Response to forskolin in the jejunum and ileum of BBdp rats.
Data are presented as mean ± SEM
Jejunum: HVβG (n=10), LVβG (n=9), barley (n=10), oatmeal (n=12), cellulose (n=12)
Ileum: HVβG (n=10), LVβG (n=8), barley (n=11), oatmeal (n=10), cellulose (n=11)

4.6.3. Correlation between the amount of β-glucan in the diets and permeability

As with the BBn rats, the association between the amount of β-glucan versus mannitol flux and the electrophysiological measures was evaluated. The association between the amount of β-glucan and mannitol flux was significant in the ileum (r=0.342, p=0.018) (Figure 18).

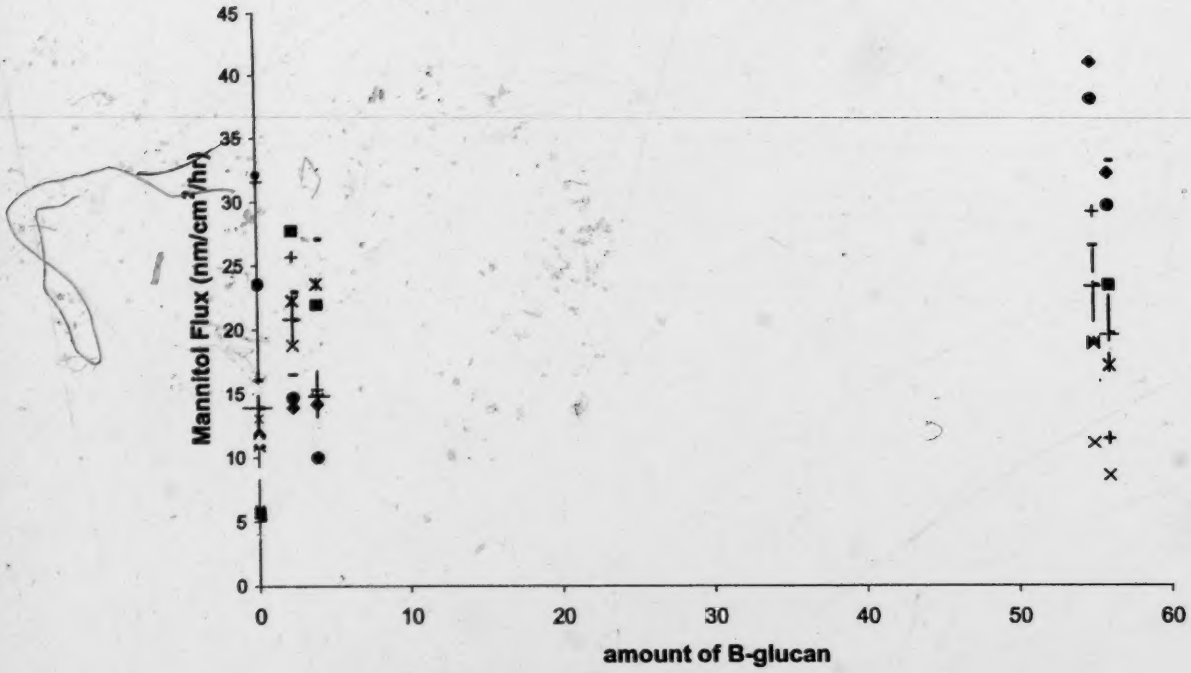

Figure 18. Correlation between the amount of β-glucan in the diet and mannitol flux.

4.7. Morphology of the ileum

Diet had no effect on villus height (p=0.871) (Table 16). The length of the villi ranged from 364 to 386 μm with the cellulose-fed rats having the shortest villi and the HVβG group had the longest villi, but the differences were not statistically different (p=0.871). Crypt depth was also not affected by the diet (p=0.120) that the rats consumed (Table 16).

Table 16. Villus height and crypt depth of the ileum in BBdp rats.[1]

Diet	Villus Height (μm)	Crypt Depth (μm)
HVβG	386 ± 15.0 (6)	137 ± 4.65 (6)
LVβG	369 ± 7.29 (8)	135 ± 2.35 (8)
Barley	373 ± 13.1 (8)	142 ± 3.18 (9)
Oatmeal	370 ± 17.4 (10)	139 ± 1.68 (13)
Cellulose	364 ± 6.22 (8)	146 ± 3.98 (9)

1. Data are presented as mean ± SEM (n)

4.8. Immunohistochemistry staining for IgA

The number of IgA positive cells per crypt was not affected by diet (p=0.186), but the HVβG group had the highest number and the barley group had the lowest (Table 17).

Table 17. Number of IgA positive cells in the lamina propria of the ileum in BBdp rats.[1]

Diet	Number of IgA+ cells / villi
HVβG	4.6 ± 0.48 (7)
LVβG	3.5 ± 0.25 (8)
Barley	3.3 ± 0.18 (9)
Oatmeal	3.7 ± 0.52 (8)
Cellulose	3.9 ± 0.17 (8)

1. Data presented as mean ± SEM (n)

Chapter 5
Discussion

5.1. Overview of project

The GI tract is the first place where food interacts with the body and thus has an important role in the overall health of an individual (Schneeman 2002). Dietary ingredients such as fibre may affect the barrier function of the intestine. β-Glucan is a cell wall polysaccharide found primarily in barley and oats and has been shown to improve cholesterol and glucose concentrations in humans and animal models. However, less is known about the effects of β-glucan on intestinal permeability and morphology. This study evaluated the effects of adding different types (alkali extracted versus whole grains) and viscosities (high or low) of β-glucan on intestinal permeability and morphology in BioBreeding normal (BBn) and diabetes-prone (BBdp) rats. At the highest concentrations of β-glucan (56g/kg), the permeability of the ileum was increased in BBn and in BBdp rats. The amount of β-glucan in the diet was positively associated with the permeability of the ileum in BBn rats ($r=0.515$, $p<0.001$) and in BBdp rats ($r=0.342$, $p=0.018$).

5.2. BBn rats

5.2.1. Intestinal Permeability

The permeability of the ileum was increased in BBn rats fed the high purity low viscosity (LVβG) diet. The permeability of the ileum observed in the LVβG group was approximately 2 fold greater than the permeability of the cellulose, oatmeal, and barley groups. The HVβG group was intermediate between the LVβG and the whole grain groups. Overall, in BBn rats, permeability of the ileum is positively associated with the amount of β-glucan in the diet (see Figure 10).

This increased permeability that was observed after rats consumed the LVβG diet (56g β-glucan / kg) has not been previously reported. Most other studies in the literature evaluating fibre have not measured intestinal permeability using the Ussing chamber technique. Shiau and Chang, (1986) fed 77 day old rats a fibre-free diet or the basal diet supplemented with guar gum, pectin, carrageenan type 1, carrageenan type 2 or cellulose at a concentration of 5% or 15% (w/w) for 28 days. Intestinal permeability was detected using urinary recovery of a phenol red dye that is poorly absorbed. At the end of the 28 days and in all of the groups, the amount of phenol red in the urine decreased from measurements taken two weeks before. All of the fibre-containing diets reduced apparent intestinal permeability with the carrageenan type I and II, or the cellulose diets having the greatest effects during the first 14 days. However, urinary recovery of phenol red dye in rats fed the pectin or guar gum (5% or 15% w/w) containing diet was not different from the fibre-free diet after 28 days of feeding. Thus, generally fibre reduces intestinal permeability, but some fibres may have a more of an effect than others. Elsenhans and

Caspary (1989) fed Wistar rats one of five diets for 4 weeks. Four diets were made by supplementing the fibre-free control diet with 20% (w/w) of pectin, guaran, methylcellulose, or carrageenan. The recovery of PEG markers in the urine was measured to assess apparent permeability of the intestine. Results using this *in vivo* technique indicated that the intestinal permeability was increased in the rats fed the viscous pectin and guaran gelling agents which are degraded by intestinal microflora (Elsenhans and Caspary, 1989). However, the specific site of increased permeability could not be indicated. These data therefore suggest that fibre fermented by bacteria may affect the barrier function of the intestine.

Some investigators have suggested that fibre may improve intestinal barrier function after injury. Rehman et al., (2003) showed that gum arabic, a soluble fibre, was able to restore potassium transport that had been disrupted by adding a deconjugated bile acid compound, chenodeoxycholic acid, to the perfusion solution. Gum arabic also improved sodium transport that had been altered by protamine, an agent that blocks the functions of tight junctions. In other study, pigs with an enteric infection that were fed an enteral diet supplemented with fructooligosaccharides (FOS) prevented the decrease in resistance (i.e. maintained barrier function) associated with an infection (Correa-Matos et al., 2003). In a rat model of chemotherapy, Deng et al., (1999) fed rats one of three diets, an enteral diet, an enteral diet supplemented with 2% (w/v) fibre manufactured from pectin, or chow for eight days. On the fourth day, rats were given 5-fluorourcil to induce intestinal damage. Intestinal permeability was assessed by measuring the urinary recovery of lactulose and mannitol on day 3 and 7. The investigators also measured bacterial translocation to the MLN. By using these direct and indirect methods,

supplementing the enteral diet with fibre was shown to prevent increases in the lactulose: mannitol ratio and the translocation of bacteria to the MLN (Deng et al., 1999). Therefore, supplementing an enteral diet with fibre derived from pectin helped maintain the intestinal barrier even after intestinal injury was established.

However, Mineo et al., (2004) evaluated the effectes of adding indigestible sugars (disaccharides or oligosaccharides) to solution on intestinal permeability and on calcium (Ca), magnesium (Mg), and zinc (Zn) absorption. The sugars that were used consisted of melibiose which is a diasaccharide of galactose and glucose; DFA II, a 'difructose anhydride'; and DFA IV, a disaccharide of fructose isomers. The intestines of 6-week old rats were excised and mounted in Ussing chambers. Investigators found that adding melibiose, DFA III or DFA IV increased the permeability of the jejunum and colon, the jejunum, ileum, and colon, or the jejunum, ileum, cecum, and colon, respectively. This increase in permeability was positively associated with nutrient (Ca, Mg, Zn) transport. The sugars also reduce transepithelial electrical resistance, which was inversely associated with Ca, Mg, and Zn absorption (Mineo et al., 1994). Therefore results from this studies shows that sugars are able to increase the permeability of the intestine in young healthy rats.

There are several possible mechanisms that could explain the increased permeability of the ileum in our study. The first explanation involves the nature of the β-glucan in the diet. Oatmeal and barley grains naturally contain β-glucan and hence contribute to the presence of β-glucan in the diet when these grains are consumed. In addition these grains contain other substances such as resistant starch, proteins, and minerals (Wood, 1990;Wood, 1993). The β-glucan in the HVβG and LVβG diets has

been extracted from whole grain barley flour, processed, and then added to the diet. Separating β-glucan from its starch and protein may alter the effects of β-glucan on the intestine. Alternatively, the starch and protein may alter the functional aspects of β-glucan (Lund and Johnson, 1991). Therefore, the presence of other nutrients may affect the behaviour of the β-glucan in the GI tract.

Viscosity of the β-glucan extract could have been a factor affecting the function and morphology of the intestine. Viscosity is the distinguishing factor between the HVβG and LVβG diet, yet there were no statistically significant differences ($p>0.05$) between the two β-glucan fractions for any of the measurements assessed in this study.

Limited data exist on the direct effects of viscosity on intestinal permeability. There are some studies that measured the effects of viscosity on various intestinal parameters that could indirectly affect permeability. For example, increased viscosity of the hydroxyethyl cellulose was associated with an increased length of the small intestine (Elsenhans and Caspary, 2000). Gee et al., (1996) also found that the length of the small intestine was longer in rats fed guar gum, a soluble fermentable fibre. Viscosity was an important characteristic that affected proliferation in the crypts of the distal ileum suggesting that viscosity may affect intestinal morphology. Altering morphology by increasing villus height and crypt depth may increase the surface area, which could provide more area for a permeant to pass through the intestine.

Viscosity of the fibre may affect the UWL. (Flourie et al., 1984) found that the UWL of the jejunum thickened in response to increasing concentration of pectin in the perfusion solution. Increasing the concentration of pectin resulted in a viscous solution which was also associated with a reduction in glucose absorption (Flourie et al., 1984).

Other researchers have proposed that viscous fibre increase the viscosity of luminal contents reducing the absorption rate of some nutrients (Battilana et al., 2001; Jenkins et al., 2002b; Vuksan et al., 2000;Wursch and PiSunyer, 1997). Viscosity may therefore affect the absorption of nutrients. The effect of viscosity on UWL may provide an indirect association between viscosity of fibre and intestinal permeability.

In our studies, we measured permeability using the Ussing chamber technique but did not measure the absorption of nutrients. The viscosity of the β-glucan in the HVβG diet is higher than that of the LVβG diet (230mPa sec vs 22mPa sec, respectively). However, we did not find a difference in permeability between the two diets. Possibly, the effects of viscosity on permeability, if any, are damped by excising the intestine and measuring intestinal permeability using an *in vitro* technique. Viscosity may not preferentially affect the barrier function of the intestine, but may affect morphology or other intestinal parameters, thereby indirectly affecting permeability *in vivo*. Furthermore, other factors or characteristics (e.g. the presence of starch or other nutrients) in the diets may contribute to the results.

The amount of β-glucan is the major difference among the diets. The cellulose, oatmeal, and barley diets contained 0g, 2.3g, and 3.9g of β-glucan / kg diet, respectively. Permeability of the ileum was similar among the HVβG and LVβG groups and higher than the diets containing the whole grains and cellulose as source of dietary fibre. Even though the HVβG and LVβG diets had the same amount of β-glucan (56g/kg), the increased permeability reached significance in the LVβG group, only. Since permeability was significantly increased only in the ileum ($p=0.003$) of the LVβG group, viscosity may have limited effects on the *in vitro* permeability of the intestine.

Another important facet involves the microflora of the intestine. The microflora can be influenced by fibre, in turn, bacteria themselves can affect intestinal barrier. For example, certain dietary fibres (e.g. FOS, lactulose, β-glucan) have been shown to affect the growth and activity of bacteria that are linked to health benefits (Blaut 2002; Dongowski et al., 2002;Kontula et al., 1998). The permeability of the colon is reduced with the presence of *Lactobacillus brevis,* a probiotic (Garcia-Lafuente et al., 1998). However, *Escherichia coli* (*E. coli*) and *Streptococcus viridans* in the GI tract may increase intestinal permeability (Garcia-Lafuente et al., 2001).

SCFAs produced by fermentation of fibre in the colon are proposed to help maintain epithelial integrity (Sakata, 1995) and increase villus height and crypt depth (Andoh et al., 1999;Goodlad et al., 1986). SCFA production could be influenced by the amount and / or type of soluble fibre that is available in the colon (Dongowski et al., 2002). Although diets in this study had the same amount of total fibre, the amounts of soluble fibre may vary among the diet groups. Cellulose, containing no soluble fibre, is not fermented in the colon. The barley and oatmeal groups inherently contain β-glucan but may also contain other dietary fibre such as resistant starch. Resistant starch has been shown to be a substrate for butyrate production (Le Blay et al., 1999;Dongowski et al., 2002). The HVβG and LVβG diets contain β-glucan that consists of soluble and insoluble fractions. Additionally, all diets contain cellulose and the amount of cellulose was higher in the barley (75g/kg) and oatmeal (75g/kg) compared to 22g/kg in both the HVβG and LVβG diets. Thus amount of soluble and fermentable fibre in the β-glucan extracts may be limited to the soluble fibre found in the structure. However, β-glucan from the isolated oat gum has been shown to be rapidly fermented in the colon (Lund and

Johnson, 1991) suggesting that the β-glucan in the diets may be fermented in the colon. Interestingly, (Mosenthal et al., 2002) showed that cellulose in an enteral diet helped maintained the barrier function.

SCFA production could also be influenced by the amount and / or species of mircoflora that are colonized in the intestine. (Dongowski et al., 2002) measured the microbial counts in feces of rats fed diets containing different amount of total fibre, β-glucan, and resistant starch. There was an increase in *lactobacillus* microbes after consuming the diets for 6 weeks, which paralleled and increase in total SCFA (acetate, propionate and butyrate).

Elsenhans and Caspary (1989) suggest that the increased urinary excretion of PEG in rats was caused by feeding a dietary fibre (i.e.pectin or guaran) that could be degraded by intestinal microflora, but feeding fibre that is resistant to bacterial degradation (i.e. methylcellulose or carrageenan) did not increase PEG excretion. An increased amount of β-glucan in the diet may be rapidly fermented in the colon affecting the microflora colonies and SCFA production. Therefore, the bacteria colonies in the colon may affect fibre fermentation and intestinal permeability, possibly through a mechanism involving SCFA. Further studies that characterize the intestinal microflora following the feeding of the different fibre used in this study may shed light on this issue.

It is possible that the increased permeability in the LVβG fed rats reflects an increase in surface area of the intestine (Dias et al., 1998;Madsen et al., 1995a). In this study, the morphology was evaluated by measuring the villus height and crypt depth. The length of the villi and the depth of the crypts of rats fed the LVβG diet were not

statistically different from any of the other diet groups ($p>0.05$) (Table 8). Therefore, the changes in permeability were unlikely to be caused by differences in surface area.

Another possible explanation involves the role of β-glucan in the excretion of bile (Lia et al., 1995). While preparing the ileal sections for the Ussing chamber, greenish-yellow gel was observed in the digesta of rats fed either the high or low viscosity β-glucan diets. The contents of this gel was not analysed but the colour suggests that bile acids may have been present.

In addition to its vital role in cholesterol metabolism, bile acids have been shown to affect the intestinal barrier. Rehman et al., (2003) and Teichberg et al., (1983) indicated that deconjugated bile acids may disrupt tight junctions and erode the mucosa, which could reduce the intestinal barrier function and allow more molecules to cross the epithelial membrane. Chadwick et al., (1979) and Gyory and Chang (1983) found a positive association between increased bile acids and colonic permeability in rabbits and in rats. Dongowski et al., (2003) found that diets based on barley products increased the amount of neutral sterols in luminal contents in rats fed diets with the highest amounts of β-glucan (60.3 and 67.8g/kg) compared to those fed diets with less than 42g β-glucan /kg diet. If β-glucan increases the fecal excretion of bile acids, more bile acids may reach the ileum and potentially disrupt the tight junctions, leading to increased permeability of the ileum.

However, a few questions arise when considering this hypothesis. One relates to the availability of bile acids to affect the intestinal mucosa. It is unknown whether β-glucan binds to the bile acids rendering them unavailable to attach to the mucosa and disrupt the tight junctions or whether β-glucan simply enhances the excretion of the bile

acids, which are available to disrupt the tight junctions. Another theory combines both concepts, suggesting that the increased amount of bile acid excreted is greater than the binding capacity of β-glucan. This results in some unbound bile acids that could disrupt tight junctions while the others are bound to β-glucan and excreted without affecting tight junctions or epithelial structure. Fermentation of fibre by the intestinal microflora was shown to increase secondary bile acid excretion (Dongowski et al., 2003). Therefore, the microflora could have a role in changing the permeability by directly affecting the mucosa or indirectly by altering bile acid excretion.

Further studies could explore the relationship among barley-derived β-glucan, bile acid excretion, and permeability of the ileum. For example, they could evaluate the amount of bile acids excreted, whether bile acids bind to the mucosa or β-glucan, pH of the luminal content, and the structure of the tight junction in the ileum of rats fed the β-glucan diets compared to a control (e.g. cellulose or barley flour).

5.2.1.1. Jejunal permeability

The diets fed to rat in this study had no effect on mannitol flux in the jejunum of BBn rats (Figure 5). Other investigators have noted changes in the ileum but not in the jejunum in response to a dietary treatment. Mosenthal et al., (2002) found that the intestinal barrier was reduced in the ileum of rats fed an enteral formula, but not in any other sections of the small intestine. Differences in adaptations among the different intestinal segments have also been suggested elsewhere (Madsen et al., 1996;Fedorak et al., 1991). Madsen et al., (1996) found that glucose transport in the ileum was normalized in streptozocin-induced diabetic rats 12-hours after insulin administration whereas it took 48-hours in the jejunum. Mariadason et al., (1999) have shown that diet influenced the

flux of ^{51}Cr-EDTA only in the distal colon and not in the jejunum or cecum (Mariadason et al., 1999). Data from Mariadason et al., (1999) implies that the jejunum may be less affected than the ileum by the by-products of fibre fermentation. Therefore, the ileum adapts differently than the jejunum to various situations.

5.2.2. Electrophysiological parameters of the small intestine

The total amount of bidirectional ion flux (I_{sc}), the maintenance of the ion concentration gradients and the Na/K ATPase activity (PD), and the ability of the ions to pass through the paracellular route (G) were not affected by the type of fibre in the diet (figures 6-8) in BBn rats. Schwartz et al., (1982) fed male rats one of three diets for four weeks. One group was fed a fibre-free control diet; the other two groups were fed the control diet supplemented with either 10% (w/w) cellulose or a combination of 5% (w/w) pectin and 5% (w/w) cellulose. With the Ussing chamber, they measured ion fluxes and the electrophysiological parameters of the jejunum. They found that the net sodium and chloride fluxes were reduced in both the cellulose- and the pectin + cellulose-containing diets relative to the fibre-free diet (Schwartz et al., 1982). Compared to the fibre-free diet, the PD and I_{sc} were lower in the diets containing fibre regardless of the type of fibre. Therefore, these results concur with the lack of differences seen in the PD, I_{sc} and G readings among the diet groups in our data that contained the same amount of fibre but was derived from different sources. Mariadason et al., (1999) found that adding wheat bran or methylcellulose did not affect G in the jejunum or cecum but did affect G in the distal colon.

5.2.3. IgA positive cells

In order to help protect the host from pathogens and foreign antigens present in the luminal contents, the GALT produces IgA to bind the pathogen, rendering it ineffective (van Egmond et al., 2001). Therefore, the immune system is activated when the body perceives a threat and the plasma cells in the LP of the intestine produce IgA (Brandtzaeg et al., 1985). Counting the number of IgA positive cells in the LP of the intestine can therefore indicate whether the immune system had been activated or not. Other studies in our laboratory have shown that a fermentable fibre, inulin, did not increase the number of IgA positive cells compared to the BBn rats fed the cellulose diet (Stillie, 2003). Our study concurs with these observations since the number of IgA positive cells in the LP was not different among the different diets. The number of IgA positive cells in the BBn rats fed the LVβG diet was similar to those of rats fed barley, oatmeal or cellulose diet (Table 11) who had lower permeability of the ileum. Therefore, our data suggests that neither the diet nor the increased permeability of the ileum was associated with an increase the production of IgA positive cells.

5.2.4. Intestinal length and morphology

Intestinal length of BBn rats fed the HVβG diet was elongated compared to those fed the barley, oatmeal, or cellulose diets, but was similar to the LVβG group. The length of the intestine of the LVβG group was longer than that of rats fed the cellulose diet. No differences were observed among the barley, oatmeal or cellulose groups (Table 8). Studies in our laboratory have shown that feeding inulin increased the length of the intestine compared to the cellulose-fed BBn rats. Elsenhans and Caspary (1989) who

found that the length of the intestine of rats fed fibre (pectin, guaran, methylcellulose, or carrageenan) was longer than those eating the fibre-free diet, but did not differ among the fibre-containing groups.

It was unexpected that there were no differences in villus height or crypt depth observed among diets in this study. Increased villus height and crypt depth have been reported in several studies evaluating the effects of soluble fibre (e.g. pectin, inulin) versus a diet without fibre or with insoluble fibre (Andoh et al., 1999; Goodlad et al., 1986; Tasman-Jones et al., 1982;Stillie, 2003). Sigleo et al., (1984) have shown that fibre (i.e. cellulose or pectin) increased jejunal villus height compared to a fibre-free control diet. Results from Sigleo et al., (1984) indicate that adding fibre to the diet, regardless of its source, increases villus height compared to a fibre-free diet. Our results however disagree with Andoh et al., (1999) who found that adding 2.5% pectin to the control diet compared to a fibre-free (i.e. control) diet increased villus height and crypt depth of the jejunum and ileum. However, Andoh et al. (1999) compared diets containing fibre to diets without any fibre and they did not observe differences among the different types of fibre on intestinal morphology.

A few explanations may explain the lack of difference in villus height. β-Glucan has both soluble and insoluble components resulting in differences in the quantity of soluble fibre in the diets. A low amount of soluble fibre may not stimulate changes in morphology seen with studies that have used only soluble fibre (McCullough et al., 1998). In the study by Stillie (2003) in which inulin, a FOS, was added to the diet at approximately 4.8% (w/w) of total fibre villus height increased compared to rats fed the diet containing cellulose (Stillie, 2003). The amount of soluble fibre in the inulin diet

may have been higher than the amount in the diets containing the β-glucan fractions. Fermentation of soluble fibre produces SCFA (Sakata, 1995) which have been associated with epithelial cell proliferation causing a trophic effect on the intestine of rats (Andoh et al., 1999; Klurfeld, 1999).

A second possible explanation relates to variation in the adaptation of different sections of the intestine. As previously mentioned, the ileum adapts differently than the jejunum to glucose transport (Madsen et al., 1996). Feeding FOS to BBn rats resulted in an increased jejunal villi height (Stillie, 2003), however, the effects in the ileum were not measured.

A third reason revolves around the morphology measurements and difficulty in assessing some of the samples. Damage to the villi may have occurred during collecting and processing of the sample leaving a relatively small number of villi to be measured. It is difficult to assess whether the villi that were "countable" were truly representative of all villi in the ileum.

A fourth reason involves the timing and exposure of the animals to the different fibre sources. During the 2-week feeding trial, the rats in the treatment groups were exposed for the first time to a soluble or fermentable fibre because all dams and their pups were fed the cellulose diet until weaning. It is possible that a 2-week period exposure to small amounts of soluble fibre and no prior exposure during lactation may have been insufficiently long to induce changes in the morphology of the ileum. The lack of dietary effect on morphology in the ileum seen in our study is likely to be a combination of many factors including the sample processing, amount and type of fibres, how they interact with

the different sections of the intestine, the timing of exposure and differential effects of the fibre in different parts of the intestine.

5.2.5. Body weight and intake

Final body weight and weight gain of HVβG-fed female rats were significantly less than the oatmeal group (Table 6). In male rats, diet had no effect on final body weight. The differences in weight gain paralleled food intake for female rats. Food intake was lower in the HVβG group compared to those fed the oatmeal diet.

5.2.6. Spleen weight

The weight of the spleen was included to show that the type of fibre in the diets did not adversely affect the growth of other organs. As shown in table 9, the HVβG-fed rats had lighter spleens than those in the barley and oatmeal groups, but had similar spleen weights to the LVβG and cellulose groups in both male and female rats. There were no observations (behaviour, coat) that would indicate adverse health effects of the diet on the animal.

5.3. BBdp rats

5.3.1. Intestinal permeability

The amount of β-glucan in the diet was associated with permeability of the ileum ($r=0.324$, $p=0.018$) (Figure 18). In the BBdp model with an altered intestinal permeability, the fibre used in the study had minimal, if any, effect of ileal permeability ($p=0.047$). The LVβG fibre used in this study had no significant effects on permeability ($p\geq0.513$). Meanwhile, the HVβG fibre tended to increase permeability relative to cellulose ($p=0.066$) group, but the effect was not significant. The data suggests that fibre may help ameliorate injury induced by exogenous agents or pathogens but has dampened effects with inherent intestinal abnormalities.

In the BB rat model, Meddings et al., (1999) showed an increase in the gastric and upper small intestine permeability of BBdp rats fed chow or a modified casein hydrolysate diet. The gastric and intestinal permeability was increased at approximately 50 days of age, which was prior to the onset of diabetes. In the study by Meddings et al. (1999), no differences in intestinal permeability were observed among diet groups. Differences in the age of the rats and the method used to measure permeability may explain some of the discrepancies between results from our studies and those reported by Meddings et al (1999). There is a tendency of the HVβG diet to increase the permeability of the ileum compared to the cellulose diet, yet this difference did not reach statistical significance ($p=0.066$). Therefore, the differences found in BBn rats were not observed in BBdp rats.

Reimer et al., (1997) found that the intestines of BBdp rats differ from those of BBn rats. BBdp had a higher amount of proglucagon mRNA in the ileum and of glucagon-like peptide-1 (GLP-1) in the plasma than did the BBn rats (Reimer et al., 1997). However, they found that the colonic proglucagon mRNA was lower in BBdp versus BBn rats (Reimer et al., 1998). The intestinal length of BBdp did not grow in response to consuming fermentable fibre as it did in the BBn rats (Stillie, 2003). It has been suggested that the intestine of BBdp and BBn rats differ, which may contribute to the onset of disease in the BBdp rats (Reimer et al., 1998) and may reflect the different observations for permeability between the two strains of rats.

5.3.2. Electrophysiological parameters

G, I_{sc}, and PD did not differ among diet groups in the BBdp rats ($p>0.05$) (Figures 14-16). In non diseased animals, the type of fibre has been shown to be ineffective at changing I_{sc} and PD readings of Sprague-Dawley rats (Schwartz et al., 1982). Although, diabetes may increase I_{sc} readings compared to non diabetic rats (Fedorak et al., 1987), animals in our study did not display overt diabetes. at the age tested. Thus, it appears that changes in the electrophysiological properties are not altered at this age (35 ± 2 days of age). The G, I_{sc}, and PD were not affected by diet.

The response of the tissue to an adenyl cyclase activator, forskolin, was increased in rats fed the oatmeal diet compared to the barley group (Figure 17). Implications of this finding are unclear but could reflect an improved *in vitro* viability of the tissue from rats fed the oatmeal diet. The increased forskolin response may also represent an increased in ion transport, the majority of which is chloride (Barrett and Keely, 2000;Walker et al.,

2003). Increasing chloride secretion would be followed by an increase in water absorption, which would help to hydrate the mucosa (Barrett and Keely, 2000) and contribute to maintaining or improving the barrier function.

5.3.3. IgA positive cells

The number of IgA positive cells in the LP was not significantly different among the diet groups in the BBdp rats. The HVβG group tended to have slightly more IgA positive cells than that of the barley, LVβG, oatmeal, and cellulose groups (Table 17). The number of IgA positive cells in the LP was comparable among the diet groups indicating that the type of fibre did not stimulate the immune system.

5.3.4. Intestinal length and morphology

The intestinal length of rats fed the cellulose-containing diet was slightly shorter than the barley and HVβGgroups. However, when taking into account the final body weights, the effect of diet disappears (Table 14). These results are similar to Stillie (2003) who found that the length of the small intestine did not differ between the BBdp rats fed the inulin or cellulose diets. Villus height and crypt depth were not affected by the type or the viscosity of the fibre in this study. Stillie (2003) showed that feeding inulin, using a similar protocol to this study, was not associated with increased villus height or changes in crypt depth in BBdp rats.

5.3.5. Body weight and food intake

Body weight of BBdp rats were similar among the diet groups (p=0.122) (Table 12). However, food intake was lower in the HVβG and LVβG compared to the cellulose and barley groups (Table 13). The rats fed the HVβG diet ate about 32g less than the cellulose group during the 14-day feeding trial, but gained about the same amount of weight (p=0.448) as the cellulose-fed rats. Thus rats fed the diet containing the β-glucan extracts were able to compensate for the difference in food intake. Fermentation of the β-glucan may produce SCFA and provide energy. Gut length was increased in the group fed the HVβG diet; however, when gut length is expressed per unit of body weight, there are no longer differences among the diet groups. Therefore, it is unlikely that intestinal length could explain the similar body weight despite the differences in food intake.

5.3.6. Spleen weight

As observed in the BBn rats, diet did not affect the weight of the spleen in BBdp rats (Table 15). Therefore, diets did not have adverse effects on the growth of organs outside the GI tract.

5.4. Summary

Permeability of the intestine plays a crucial role in absorbing nutrients and preventing the entry of foreign antigens and pathogens into the systemic circulation. An increase in permeability was observed prior to the onset of Chron's disease (Söderholm,

1998) and type 1 diabetes in humans (Carratu et al., 1999) and in rodents (Meddings et al., 1999). Dietary fibre however has been shown to reduce intestinal permeability of animals with injuries to the intestine (Correa-Matos et al., 2003; Deng et al., 1999; Rehman et al., 2003).

Data on the effects of β-glucan, a cell wall polysaccharide, on intestinal permeability are limited. Results from this study indicate that incorporating the low viscosity alkali extract β-glucan into the diet increases the permeability of the ileum, but not the jejunum in BBn rats. These rats are not known to have altered permeability suggesting that diet has an influence on barrier function. The results indicate that diet, particularly the β-glucan, increases permeability of normal rats in the post weaning period (21 to 35 days of age) that were not previous subjected to intestinal injury. This change occurred without affecting ion status of the tissue, villus height, crypt depth, and number of IgA positive cells in the lamina propria. It is unknown what long-term consequences may arise due to this increased permeability of the ileum. Furthermore, the effect of diet on intestinal permeability was dampened in the BBdp rats; the high viscosity β-glucan tended to increase permeability of the ileum relative to rats fed the cellulose diet.

Therefore, the increased permeability of the ileum was not associated with an increased production of IgA positive cells indicating that the mucosal immune system was not stimulated by the presence of the fibre. The current data suggest that there might be several outcomes caused by increased permeability. The general idea is that increased intestinal permeability is pathological and associated with diseases or injuries to the intestine. However, there might be an increased permeability in the early post weaning

period where the body adapts to the numerous challenges or diet may affect barrier function without compromising the immune system.

5.5. Future research

Several questions have been raised by these studies, notably in the explanation for the increased permeability. The results showed that the permeability of the ileum is increased in rats fed diet containing the highest amount of β-glucan. β-glucan is known to increase excretion of bile acids (Lia et al., 1995) and bile acids have been suggested to disrupt tight junctions (Rehman et al., 2003;Teichberg et al., 1983). Therefore, studies on assessing the amount of bile acids excreted, whether bile acids binds to β-glucan or the mucosa, or the structure of tight junctions are warranted. It may be worthwhile to determine the long-term consequences, if any, of the increased permeability.

Another area of interest involves the solubility and fermentation of β-glucan and their effects on the intestine. In the larger picture, solubility of β-glucan may affect cholesterol and glucose metabolism. Results indicated that viscosity may moderate the effects of β-glucan on the intestinal permeability, but does not fully explain the observations. The amount of soluble component in β-glucan may vary and perhaps differences in the amounts of soluble fibre could affect intestinal morphology. Fermentation of the β-glucan may also occur. In order to assess whether the solubility of β-glucan affect the intestine, diets should be supplemented with β-glucan extracts containing various amount of soluble fibre and measure intestinal absorption of nutrients. Furthermore, measurements in the periphery such as lipid profile and postprandial blood

glucose concentration may provide insight on the effects of solubility of barley and oat-derived β-glucan effect on the body.

Appendix 1

Overview of Barley Project

β-Glucan has been proposed to help with lipid and glucose metabolism (Braaten et al., 1994b, 1994a), yet most studies used β-glucan derived from oats. In the USA, oat products may carry a health claim associating the consumption of oats and reductions in cholesterol concentrations and CVD risk. Fewer studies evaluated β-glucan the role of barley-derived. Dr. Temelli and Dr. Vasanthan, food Scientists at the University of Alberta, have developed a method to extract the β-glucan from barley. These extracts were tested on cell lines for the expression of cholesterol metabolism genes. The extracts were also fed to rats, and its effects on the immune system, lipid metabolism, microflora, nutrient absorption. The study described in this thesis is part of the larger animal trial (Figure 19).

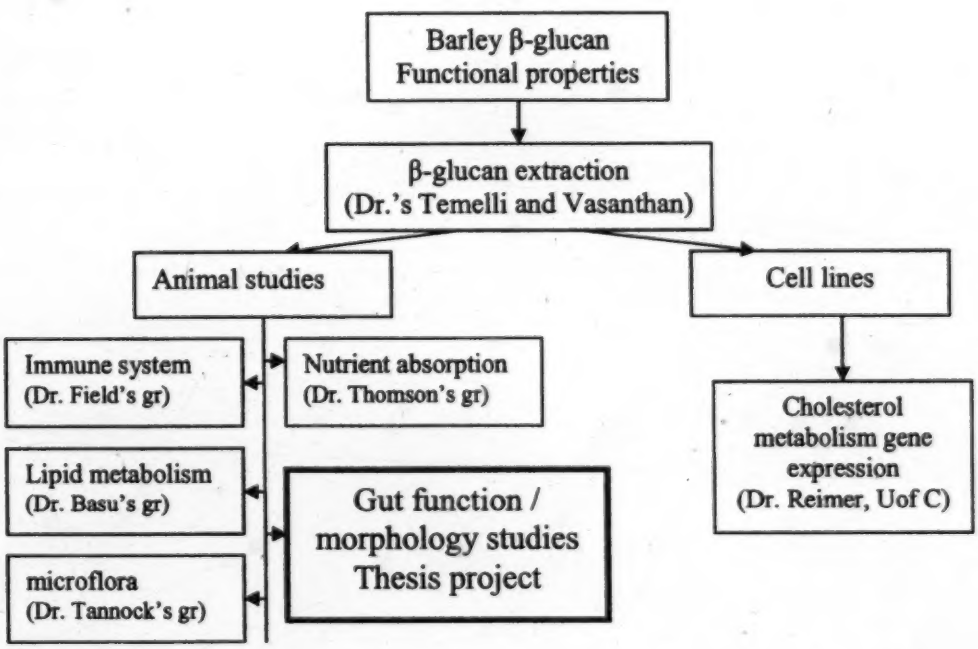

Figure 19. Overview of barley project.

Appendix 3
β-Glucan Extraction Flow Chart

β-Glucan was extracted from Candle barley flour using an alkali extraction process

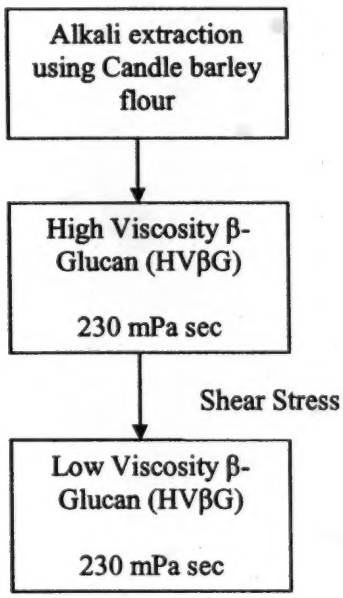

Figure 20. β-Glucan extraction flow chart

Appendix 3a

Modification of Diet

Mid way through the BBdp gut function / morphology study, the fatty acid composition of the Tosca Safflower oil changed from a high polyunsaturated (PUFA) and low monounsaturated fatty acids (MUFA) to the low PUFA and high MUFA profile. For example, the original Tosca oil provided 7.9g of PUFA and 1.4g of MUFA per 10g of fat compared to 2g from PUFA and 11g from MUFA (total fat 14g) after the switch. The original Tosca oil was used, in part, as a source of omega-6 fatty acids. Table 18 provides the amount (and percentage) of MUFA, PUFA, saturated (SFA) in each source of oil.

	Sources of oil			
Type of Fat g (%)	Linseed	Tosca (original)	Tosca (modified)	Safflo
MUFA	3 (21.43)	1.4 (14)	11 (78.57)	1.5 (16)
PUFA	10 (71.43)	7.9 (79)	2 (14.29)	6.5 (70)
SFA	1 (7.14)	0.7 (7)	1 (7.14)	1.2 (14)
Total	14 (100)	10 (100)	14 (100)	9.2 (100)

Table 18. Sources of oil and fatty acids. Table provides the amount in g and the (percent of total fat) for the amount of MUFA, PUFA, SFA, in each oil. Composition is based on the nutrition information panel.

The amounts of MUFA, PUFA, and SFA in 1kg of diet made with the different oils are listed in table 19. The total amount of PUFA in 1kg diet decreased about 40g from the original to modified Safflower oil. The quantities were calculated by multiplying the percentage each type of fat times the amount added to the diet. For example linseed, 79% is PUFA and 7.4g was used, thus 0.79 * 7.4g = 5.29g PUFA/kg diet provided by linseed. Total amount of PUFA was calculated by adding all sources of PUFA in the diet.

Type of Fat g	Sources of oil				
	Soybean stearine	Linseed	Tosca (original)	Tosca (modified)	Safflo
MUFA	-	1.59	8.68	48.71	10.37
PUFA	-	5.29	48.98	8.86	48.37
SFA	131	0.53	4.34	4.43	9.67
Total PUFA	-	5.29	54.18	14.15	53.66

Table 19. The amount (g) of each type of fat (MUFA, PUFA, SFA) in 1kg diet for each of the oil used.

Therefore, the brand of safflower oil was changed to Safflo and the amount of each oil added to each diet was adjusted (see below for calculations) to reflect and maintain the original polyunsaturated to saturated fat (P:S) ratio of 0.4.

Appendix 3b
Calculations

Calculations based on 1kg diet.
Total fat: 200.4g
Linseed oil in 1kg diet: 7.4g (this number is constant) multiply 7.4g* 0.70=g PUFA in oil;

7.4g * 0.14=g SFA

Amount of fat that needs to be shared between soybean stearine and sunflower:
=200.4g-7.4g
=193g
x= soybean stearine
y= sunflower oil
solve for x then y in order to keep a P:S ratio of 0.4

	soybean + sunflower + linseed = P:S
PUFA in oils	0x + 0.70y + 5.25 = 0.4
SFA in oils	1x 0.14y 0.52
	1 (0x + 0.7y + 5.25) = 0.4 (1x + 0.14y + 0.52)
	0x + 0.7y + 5.25 = 0.4x + 0.056y + 0.208
	0.7 (193-x) + 5.25 = 0.4x + 0.056 (193-x) + 0.208
	135.1 - 0.7x + 5.25 = 0.4x +10.808 - 0.056x + 0.208
	140.334 + 0.7x = 0.344x + 11.016
	129.334 = 1.044x
	123.9 = x

y= 193-123.9
y= 69.1

Therefore, 123.9g should come from soybean stearine and 69.1g from Safflo oil to get a P:S ratio of 0.4

Appendix 3c
Diet Formulation

Using the calculations above, modifications were made to the amount of ingredients added to the diet. The quantity of oils changed in order to keep a polyunsaturated to saturated fatty acid ratio of 0.4 and to maintain comparable macronutrient and fibre profile. The diet ingredients with the modifications made to the amount are listed in Table 20.

Table 20 (a). Amount and source of macronutrients in the diets made with modifications due to changes in oils

	Diet				
Macronutrient	HVβG	LVβG	Barley	Oatmeal	Cellulose
Total amount in diet (g)	**1016.9**	**1022.5**	**992.9**	**1033.9**	**999.9**
Total Protein (g/kg)	**270.4**	**270**	**267.3**	**268**	**270**
casein	265	270	260	268	270
β-glucan	5.4	3.9	-	-	-
barley flour	-	-	7.3	-	-
oatmeal flour	-	-	-	10	-
Total Lipids (g/kg)	**200.4**	**200.4**	**196.6**	**203.7**	**200.4**
soybean stearine	123.9	123.9	120.9	123.9	123.9
safflower	69.1	69.1	67.1	69.1	69.1
linseed	7.4	7.4	7.4	7.4	7.4
barley flour	-	-	1.2	-	-
oatmeal flour	-	-	-	3.3	-
Total Carbohydrates (g/kg)	**381.1**	**385.6**	**372.3**	**391.9**	**378**
corn starch	380	385	343	335	378
β-glucan	1.1	0.6	-	-	-
barley flour	-	-	29.3	-	-
oatmeal flour	-	-	-	56.9	-
Total Fibre (g/kg)	**77.9**	**78.1**	**79.2**	**77.3**	**80**
cellulose	22	22	76	75	80
β-glucan	55.9	56.9	-	-	-
barley flour	-	-	3.6	-	-
oatmeal flour	-	-	-	6.4	-

Table 20 (b). Summary of the percentage of macronutrients and fibre in each diet

	Diet				
Nutrient (%w/w)	**HVβG**	**LVβG**	**Barley**	**Oatmeal**	**Cellulose**
total protein	27	27	27	27	27
total lipids	20	20	20	20	20
total CHO	37.5	37.7	37.9	37.5	37.8
total fibre	7.7	7.7	8.0	7.9	8.0

*Diets are supplemented with 10g/kg of vitamin mix and 50g/kg of mineral mix

Appendix 4
Incidence trial

Purpose

An incidence trial was conducted to demonstrate that the effect of feeding a semi-purified casein-based diet on the incidence of diabetes in our BBdp rat colony. The procedure is listed below.

Animals

To determine the incidence of diabetes in the BBdp colony, four to six BBdp rats were housed per shoebox cage until 50-64 days of age and were then housed individually in hanging stainless steel metabolic cages until the diagnosis of diabetes or 120 days of age, which ever came first. Rats that were losing weight, had a rough-looking coat or had many wet spots in the bedding underneath their cages were suspected to have hyperglycemia. Hyperglycemia and diabetes were diagnosed using the following steps. The urine of suspected hyperglycemic rats was tested for glucose (Chemstrip uG/K, Roche, Laval, Canada). If glucose was found in the urine, a blood sample was taken from the tail vein and the blood glucose concentration was measured using a MediSense Precision Xtra blood glucose monitor (Abbott Laboratories, Bedford MA, USA). Rats with a blood glucose value ≥12 mmol/L were diagnosed with diabetes and killed.

Diet

Rats were fed the same cellulose diet described for gut function / morphology studies. To recapitulate, the diet is a semi-purified, casein-based containing 27% (w/w) protein, 38% (w/w) carbohydrate, 20% (w/w) lipids and 8% (w/w) fibre (ie. cellulose). Data from this incidence trial is compared to the incidence in the same colony but with rats fed chow.

Incidence calculation

The incidence of diabetes was calculated as the number of rats testing positive for diabetes divided by the total number of rats used in the trial.

Results

The incidence trial was conducted to determine the incidence of diabetes in BBdp rats fed the semi-purified diet containing cellulose as the fibre source. Eleven of the 48 (23%) BBdp rats fed the semi-purified casein-based diet developed autoimmune diabetes by 120 days of age compared to 50% in the chow-fed group (x^2= 9.91, $p<0.01$). These data indicates that the cellulose diet reduces but does not completely prevent the incidence of diabetes in the BBdp rats fed cellulose diet

Conclusion

The semi-purified casein-based diet containing cellulose as the fibre source reduces, but does not prevent the incidence of autoimmune diabetes in our BBdp rat colony.

Reference List

A report on the definition of dietary fiber. Dietary reference intakes: proposed definition of dietary fiber. Washington: National Academy Press, 2001.

Alberts DS, Einspahr J, Reesmcgee S, Ramanujam P, Buller MK, Clark L, Ritenbaugh C, Atwood J, Pethigal P, Earnest D, Villar H, Phelps J, Lipkin M, Wargovich M, Meyskens FL. Effects of Dietary Wheat Bran Fiber on Rectal Epithelial-Cell Proliferation in Patients with Resection for Colorectal Cancers. Journal of the National Cancer Institute 1990; 82:1280-1285.

Aldoori WH, Giovannucci EL, Rimm EB, Wing AL, Trichopoulos DV, Willett WC. A Prospective-Study of Diet and the Risk of Symptomatic Diverticular-Disease in Men. American Journal of Clinical Nutrition 1994; 60:757-764.

Aldoori WH, Giovannucci EL, Rockett HRH, Sampson L, Rimm EB, Willett WC. A prospective study of dietary fiber types and symptomatic diverticular disease in men. Journal of Nutrition 1998; 128:714-719.

American Dietetic Association, Dietitians of Canada. High-fiber diet. In: Manual of Clinical Dietetics. Chicago: American Dietetic Association, 2000. p. 709-717.

Anderson JW, Allgood LD, Lawrence A, Altringer LA, Jerdack GR, Hengehold DA, Morel JG. Cholesterol-lowering effects of psyllium intake adjunctive to diet therapy in men and women with hypercholesterolemia: meta-analysis of 8 controlled trials. American Journal of Clinical Nutrition 2000; 71:472-479.

Anderson JW, Randles KM, Kendall CWC, Jenkins DJA. Carbohydrate and fiber recommendations for individuals with diabetes: A quantitative assessment and meta-analysis of the evidence. Journal of the American College of Nutrition 2004; 23:5-17.

Andoh A, Bamba T, Sasaki M. Physiological and anti-inflammatory roles of dietary fiber and butyrate in intestinal functions. Journal of Parenteral and Enteral Nutrition 1999; 23:S70-S73.

Barrett KE, Keely SJ. Chloride secretion by the intestinal epithelium: Molecular basis and regulatory aspects. Annual Review of Physiology 2000; 62:535-572.

Battilana P, Ornstein K, Minehira K, Schwarz JM, Acheson K, Schneiter P, Burri J, Jequier E, Tappy L. Mechanisms of action of beta-glucan in postprandial glucose metabolism in healthy men. European Journal of Clinical Nutrition 2001; 55:327-333.

Benjamin MA, Mckay DM, Yang PC, Cameron H, Perdue MH. Glucagon-like peptide-2 enhances intestinal epithelial barrier function of both transcellular and paracellular pathways in the mouse. Gut 2000; 47:112-119.

Beyer-Sehlmeyer G, Glei M, Hartmann E, Hughes R, Persin C, Bohm V, Rowland I, Schubert R, Jahreis G, Pool-Zobel BL. Butyrate is only one of several growth inhibitors produced during gut flora-mediated fermentation of dietary fibre sources. British Journal of Nutrition 2003; 90:1057-1070.

Bjarnason I. Intestinal Permeability. Gut 1994; 35:S18-S22.

Blaut M. Relationship of prebiotics and food to intestinal microflora. European Journal of Nutrition 2002; 41 Suppl 1:I11-I16.

Bourdon I, Yokoyama W, Davis P, Hudson C, Backus R, Richter D, Knuckles B, Schneeman BO. Postprandial lipid, glucose, insulin, and cholecystokinin responses in men fed barley pasta enriched with beta-glucan. American Journal of Clinical Nutrition 1999; 69:55-63.

Braaten JT, Scott FW, Wood PJ, Riedel KD, Wolynetz MS, Brule D, Collins MW. High Beta-Glucan Oat Bran and Oat Gum Reduce Postprandial Blood-Glucose and Insulin in Subjects with and Without Type-2 Diabetes. Diabetic Medicine 1994a; 11:312-318.

Braaten JT, Wood PJ, Scott FW, Wolynetz MS, Lowe MK, Bradleywhite P, Collins MW. Oat Beta-Glucan Reduces Blood Cholesterol Concentration in Hypercholesterolemic Subjects. European Journal of Clinical Nutrition 1994b; 48:465-474.

Bragg LE, Thompson JS, Rikkers LF. Influence of Nutrient Delivery on Gut Structure and Function. Nutrition 1991; 7:237-243.

Brandtzaeg P. Immunology of the human gastrointestinal tract in health and disease-a brief review. Keio J Med 1988; 37:168-176.

Brandtzaeg P, Valnes K, Scott H, Rognum T. The human gastrointestinal secretory immune system in health and disease. Scandinavian Journal of Gastroenterology 1985; 20:17-38.

Briggs GM, Spiller GA. Dietary Fiber Or Plantix. Lancet 1977; 2:563.

Brighenti F, Casiraghi MC, Canzi E, Ferrari A. Effect of consumption of a ready-to-eat breakfast cereal containing inulin on the intestinal milieu and blood lipids in healthy male volunteers. European Journal of Clinical Nutrition 1999; 53:726-733.

Brown L, Rosner B, Willett WW, Sacks FM. Cholesterol-lowering effects of dietary fiber: a meta-analysis. American Journal of Clinical Nutrition 1999; 69:30-42.

Bruewer M, Luegering A, Kucharzik T, Parkos CA, Madara JL, Hopkins AM, Nusrat A. Proinflammatory cytokines disrupt epithelial barrier function by apoptosis-independent mechanisms. Journal of Immunology 2003; 171:6164-6172.

Burkitt D. Dietary fiber: from early hunter-gatherers to the 1990's. In: Spiller G, editor. Dietary fiber in Human Nutrition. Boca Raton: CRC Press, 1993. p. 3-6.

Burrin DG, Petersen Y, Stoll B, Sangild P. Glucagon-like peptide 2: a nutrient-responsive gut growth factor. J Nutr. 2001; 131:709-712.

Cameron HL, Yang PC, Perdue MH. Glucagon-like peptide-2-enhanced barrier function reduces pathophysiology in a model of food allergy. American Journal of Physiology-Gastrointestinal and Liver Physiology 2003a; 284:G905-G912.

Cameron IL, Hardman WE, Heitman DW, Carter JW. Dietary fibre on cell proliferation in large bowel mucosal crypts near or away from lymphoid nodules and on mineral bioavailability. Cell Proliferation 2000; 33:367-379.

Cameron-Smith D, Collier GR, O'Dea K. Effect of soluble dietary fibre on the viscosity of gastrointestinal contents and the acute glycaemic response in the rat. British Journal of Nutrition 1994; 71:563-571.

Carratu R, Secondulfo M, de Magistris L, Iafusco D, Urio A, Carbone MG, Pontoni G, Carteni M, Prisco F. Altered intestinal permeability to mannitol in diabetes mellitus type I. Journal of Pediatric Gastroenterology and Nutrition 1999; 28:264-269.

Chadwick VS, Gaginella TS, Carlson GL, Debongnie JC, Phillips SF, Hofmann AF. Effect of Molecular-Structure on Bile Acid-Induced Alterations in Absorptive Function, Permeability, and Morphology in the Perfused Rabbit Colon. Journal of Laboratory and Clinical Medicine 1979; 94:661-674.

Charalampopoulos D, Wang R, Pandiella SS, Webb C. Application of cereals and cereal components in functional foods: a review. International Journal of Food Microbiology 2002; 79:131-141.

Clemente MG, De Virgiliis S, Kang JS, Macatagney R, Musu MP, Di Pierro MR, Drago S, Congia M, Fasano A. Early effects of gliadin on enterocyte intracellular signalling involved in intestinal barrier function. Gut 2003; 52:218-223.

Colgan SP, Resnick MB, Parkos CA, Delp-Archer C, McGuirk D, Bacarra AE, Weller PF, Madara JL. IL-4 directly modulates function of a model human intestinal epithelium. J Immunol. 1994; 153:2122-2129.

Correa-Matos NJ, Donovan SM, Isaacson RE, Gaskins HR, White BA, Tappenden KA. Fermentable fiber reduces recovery time and improves intestinal function in piglets following Salmonella typhimurium infection. Journal of Nutrition 2003; 133:1845-1852.

Cui S. Cereal non-starch polysaccharides I; (1–>3)(1–>4)-ß-D-glucans. In: Cui S, editor. Polysaccharide Gums From Agricultural Products, Processing, structures & functionality. Lancaster: Technomic Publishing Co., 2001. p. 103-166.

Cummings J, Macfarlane G. The effect of dietary fiber on fecal weight and composition. In: Spiller G, editor. Dietary Fiber in Human Nutrition. Boca Raton: CRC Press, 1993. p. 263-349.

Danielson AD, Newman RK, Newman CW, Berardinelli JG. Lipid levels and digesta viscosity of rats fed a high-fiber barley milling fraction. Nutrition Research 1997; 17:515-522.

Davidson M, Dugan L, Burns J, Bova J, Story K, Drennan K. The hypocholesterolemic effects of beta-glucan in oatmeal and oat bran. A dose-controlled study. JAMA 1991; 265:1833-1839.

Delany B, Carlsonb T, Frazera S, Zhengb T, Hessa R, Ostergrena K, Kierzeka K, Hawortha J, Knutsona N, Junkerc K, Jonkerc D. Evaluation of the toxicity of concentrated barley b-glucan in a 28-day feeding study in Wistar rats. Food and Chemical Toxicology 2003; 41:477-487.

Deng GY, Liu YW, He GZ, Jiang ZM. Effect of dietary fiber on intestinal barrier function of 5-Fu stressed rats. Research in Experimental Medicine 1999; 199:111-119.

Di Leo V, D'Inca R, Diaz-Granado N, Fries W, Venturi C, D'Odorico A, Martines D, Sturniolo GC. Lactulose/mannitol test has high efficacy for excluding organic causes of chronic diarrhea. American Journal of Gastroenterology 2003a; 98:2245-2252.

Dias VC, Madsen KL, Mulder KE, Keelan M, Yatscoff RW, Thomson ABR. Oral administration of rapamycin and cyclosporine differentially alter intestinal function in rabbits. Digestive Diseases and Sciences 1998; 43:2227-2236.

Dongowski G, Huth M, Gebhardt E. Steroids in the intestinal tract of rats are affected by dietary-fibre-rich barley-based diets. British Journal of Nutrition 2003; 90:895-906.

Dongowski G, Huth M, Gebhardt E, Flamme W. Dietary fiber-rich barley products beneficially affect the intestinal tract of rats. Journal of Nutrition 2002; 132:3704-3714.

Duffus C, Coohrane M. Formation of the barley grain morphology, physiology, and biochemistry. In: MacGregor A, Bhatty R, editors. Barley Chemistry and Technology. Minnesota: American Association of Cereal Chemists, 1993.

Edouard P, Hiserodt JC, Plamondon C, Poussier P. Cd8+ T-Cells Are Required for Adoptive Transfer of the Bb Rat Diabetic Syndrome. Diabetes 1993; 42:390-397.

Elsenhans B, Caspary WF. Differential Changes in the Urinary-Excretion of 2 Orally-Administered Polyethylene-Glycol Markers (Peg-900 and Peg-4000) in Rats After Feeding Various Carbohydrate Gelling Agents. Journal of Nutrition 1989; 119:380-387.

Elsenhans B, Caspary WF. Food viscosity as determinant for adaptive growth responses in rat intestine: long-term feeding of different hydroxyethyl celluloses. British Journal of Nutrition 2000; 84:39-48.

Farhadi A, Banan A, Fields J, Keshavarzian A. Intestinal barrier: An interface between health and disease. Journal of Gastroenterology and Hepatology 2003; 18:479-497.

Fedorak RN, Chang EB, Madara JL, Field M. Intestinal Adaptation to Diabetes - Altered Na-Dependent Nutrient Absorption in Streptozocin-Treated Chronically Diabetic Rats. Journal of Clinical Investigation 1987; 79:1571-1578.

Fedorak RN, Thomson ABR, Porter VM. Adaptation of Intestinal Glucose-Transport in Rats with Diabetes-Mellitus Occurs Independent of Hyperphagia. Canadian Journal of Physiology and Pharmacology 1991; 69:1143-1148.

Ferraris RP, Carey HV. Intestinal transport during fasting and malnutrition. Annual Review of Nutrition 2000; 20:195-219.

Field CJ, McBurney MI, Massimino S, Hayek M, Sunvold G. The fermentable fiber content of the diet alters the function and composition of canine gut associate lymphoid tissue. Veterinary Immunology and Immunopathology 1999; 72:325-341.

Flourie B, Vidon N, Florent C, Bernier JJ. Effect of Pectin on Jejunal Glucose-Absorption and Unstirred Layer Thickness in Normal Man. Gut 1984; 25:936-941.

Food and Nutrition Board, Institute of Medicine. Dietary, functional, and total fibre. In: Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients) (2002). Washington: National Academy Press, 2002. p. 265-334.

Franz MJ, Bantle JP, Beebe CA, Brunzell JD, Chiasson JL, Garg A, Holzmeister LA, Hoogwerf B, Mayer-Davis E, Mooradian AD, Purnell JQ, Wheeler M. Evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications. Diabetes Care 2002; 25:148-198.

Friedel D, Levine GM. Effect of Short-Chain Fatty-Acids on Colonic Function and Structure. Journal of Parenteral and Enteral Nutrition 1992; 16:1-4.

Fukunaga T, Sasaki M, Araki Y, Okamoto T, Yasuoka T, Tsujikawa T, Fujiyama Y, Bamba T. Effects of the soluble fibre pectin on intestinal cell proliferation, fecal short chain fatty acid production and microbial population. Digestion 2003; 67:42-49.

Garcia-Lafuente A, Antolin M, Crespo E, Guarner F, Malagelada JR. Improvement of colonic barrier function by colonization with lactobacilli in the rat. Gastroenterology 1998; 114:A982.

Garcia-Lafuente A, Antolin M, Guarner F, Crespo E, Malagelada JR. Modulation of colonic barrier function by the composition of the commensal flora in the rat. Gut 2001; 48:503-507.

Gee JM, LeeFinglas W, Wortley GW, Johnson IT. Fermentable carbohydrates elevate plasma enteroglucagon but high viscosity is also necessary to stimulate small bowel mucosal cell proliferation in rats. Journal of Nutrition 1996; 126:373-379.

Gonzalez-Mariscal L, Betanzos A, Nava P, Jaramillo BE. Tight junction proteins. Progress in Biophysics & Molecular Biology 2003; 81:1-44.

Goodlad RA, Lenton W, Ghatei MA, Adrian TE, Bloom SR, Wright NA. Effects of An Elemental Diet and Different Types of Dietary Fiber on Intestinal Crypt Cell Production - and Its Correlation with Plasma Enteroglucagon and Pyy Levels. Gut 1986; 27:1252.

Grotz MRW, Deitch EA, Ding JY, Xu DZ, Huang QH, Regel G. Intestinal cytokine response after gut ischemia - Role of gut barrier failure. Annals of Surgery 1999; 229:478-486.

Gyory CP, Chang GW. Effects of Bran, Lignin and Deoxycholic-Acid on the Permeability of the Rat Cecum and Colon. Journal of Nutrition 1983; 113:2300-2307.

Hardin JA, Donegan L, Woodman RC, Trevenen C, Gall DG. Mucosal inflammation in a genetic model of spontaneous type I diabetes mellitus. Can.J Physiol Pharmacol. 2002; 80:1064-1070.

Health and Welfare Canada. Report of the Expert Advisory Committee on Dietary Fibre. 1985. Ottawa, Supply and Services Canada.
Ref Type: Serial (Book,Monograph)

HHS Food and Drug Administration. Food labelling: health claims; soluble dietary fiber from certain foods and coronary heart disease. Fed.Reg 2001; 67:61773-61783.

Jaskari J, Kontula P, Siitonen A, Jousimies-Somer H, Mattila-Sandholm T, Poutanen K. Oat beta-glucan and xylan hydrolysates as selective substrates for Bifidobacterium and Lactobacillus strains. Applied Microbiology and Biotechnology 1998; 49:175-181.

Jenkins AL, Jenkins DJ, Zdravkovic U, Wursch P, Vuksan V. Depression of the glycemic index by high levels of beta-glucan fiber in two functional foods tested in type 2 diabetes. European Journal of Clinical Nutrition 2002a; 56:622-628.

Jenkins DJ, Axelsen M, Kendall CW, Augustin LS, Vuksan V, Smith U. Dietary fibre, lente carbohydrates and the insulin-resistant diseases. British Journal of Nutrition 2000a; 83 Suppl 1:S157-S163.

Jenkins DJ, Kendall CW, Marchie A, Faulkner D, Vidgen E, Lapsley KG, Trautwein EA, Parker TL, Josse RG, Leiter LA, Connelly PW. The effect of combining plant sterols, soy protein, viscous fibers, and almonds in treating hypercholesterolemia. Metabolism 2003; 52:1478-1483.

Jenkins DJ, Kendall CW, Vuksan V. Viscous fibers, health claims, and strategies to reduce cardiovascular disease risk. American Journal of Clinical Nutrition 2000b; 71:401-402.

Jenkins DJA, Kendall CW, Vuksan V, Vidgen E, Parker TL, Faulkner D, Mehling D, Garsetti M, Testolln G, Cunnane S, Ryan M, Corey P. Soluble fiber intake at a dose

approved by the US Food and Drug Administration for a claim of health benefits: serum lipid risk factors for cardiovascular disease assessed in a randomized controlled crossover trial. American Journal of Clinical Nutrition 2002b; 75:834-839.

Jenkins DJA, Leeds AR, Gassull MA, Cochet B, Alberti KGMM. Decrease in Postprandial Insulin and Glucose Concentrations by Guar and Pectin. Annals of Internal Medicine 1977; 86:20-23.

Kanauchi O, Iwanaga T, Andoh A, Araki Y, Nakamura T, Mitsuyama K, Suzuki A, Hibi T, Bamba T. Dietary fiber fraction of germinated barley foodstuff attenuated mucosal damage and diarrhea, and accelerated the repair of the colonic mucosa in an experimental colitis. Journal of Gastroenterology and Hepatology 2001; 16:160-168.

Kerckhoffs D, Hornstra G, Mensink R. Cholesterol-lowering effect of â-glucan from oat bran in mildly hypercholesterolemic subjects may decrease when â-glucan is incorporated into bread and cookies. American Journal of Clinical Nutrition 2003; 78:221-227.

Klurfeld DM. Nutritional regulation of gastrointestinal growth. Front Biosci. 1999; 4:D299-D302.

Knudsen KEB. Physiological implications of wheat and oat dietary fiber. Adv Exp Med Biol 1990; 270:135-150.

Kontula P, Jaskari J, Nollet L, De Smet I, von Wright A, Poutanen K, Mattila-Sandholm T. The colonization of a simulator of the human intestinal microbial ecosystem by a probiotic strain fed on a fermented oat bran product: effects on the gastrointestinal microbiota. Applied Microbiology and Biotechnology 1998; 50:246-252.

Langkamp-Henken B, Glezer JA, Kudsk KA. Immunologic structure and function of the gastrointestinal tract. Nutr.Clin.Pract. 1992; 7:100-108.

Le Blay G, Michel C, Blottiere HM, Cherbut C. Prolonged intake of fructo-oligosaccharides induces a short-term elevation of lactic acid-producing bacteria and a persistent increase in cecal butyrate in rats. Journal of Nutrition 1999; 129:2231-2235.

Li J, Kaneko T, Qin LQ, Wang J, Wang Y. Effects of barley intake on glucose tolerance, lipid metabolism, and bowel function in women. Nutrition 2003a; 19:926-929.

Li J, Kaneko T, Qin LQ, Wang J, Wang Y, Sato A. Long-term effects of high dietary fiber intake on glucose tolerance and lipid metabolism in GK rats: comparison among barley, rice, and cornstarch. Metabolism 2003b; 52:1206-1210.

Lia A, Hallmans G, Sandberg AS, Sundberg B, Aman P, Andersson H. Oat Beta-Glucan Increases Bile-Acid Excretion and A Fiber-Rich Barley Fraction Increases Cholesterol Excretion in Ileostomy Subjects. American Journal of Clinical Nutrition 1995; 62:1245-1251.

Lifschitz CH, Grusak MA, Butte NF. Carbohydrate digestion in humans from a beta-glucan-enriched barley is reduced. Journal of Nutrition 2002; 132:2593-2596.

Lim BO, Yamada K, Nonaka M, Kuramoto Y, Hung P, Sugano M. Dietary fibers modulate indices of intestinal immune function in rats. Journal of Nutrition 1997; 127:663-667.

Lund EK, Johnson IT. Fermentable Carbohydrate Reaching the Colon After Ingestion of Oats in Humans. Journal of Nutrition 1991; 121:311-317.

Lupton JR, Morin JL, Robinson MC. Barley Bran Flour Accelerates Gastrointestinal Transit-Time. Journal of the American Dietetic Association 1993; 93:881-885.

Madara JL, Anderson J. Epithelia biological priniciples of organization. In: Yamada T, Alpers D, Kaplowitz W, Laine L, Owyang C, Powell D, editors. Textbook of Gastroenterology. Philadelphia: Lippincott WIlliams & Wilkins, 2003. p. 151-165.

Madden KB, Whitman L, Sullivan C, Gause WC, Urban JF, Katona IM, Finkelman FD, Shea-Donohue T. Role of STAT6 and mast cells in IL-4-and IL-13-induced alterations in murine intestinal epithelial cell function. Journal of Immunology 2002; 169:4417-4422.

Madsen KL, Ariano D, Fedorak RN. Insulin downregulates diabetic-enhanced intestinal glucose transport rapidly in ileum and slowly in jejunum. Canadian Journal of Physiology and Pharmacology 1996; 74:1294-1301.

Madsen KL, Yanchar NL, Sigalet DL, Reigel T, Fedorak RN. Fk506 Increases Intestinal Permeability in Rats Through Inhibition of Enterocyte Mitochondrial-Function. Gastroenterology 1995a; 108:A304.

Madsen KL, Yanchar NL, Sigalet DL, Reigel T, Fedorak RN. Fk506 Increases Permeability in Rat Intestine by Inhibiting Mitochondrial-Function. Gastroenterology 1995b; 109:107-114.

Malkki Y. Physical properties of dietary fiber as keys to physiological functions. Cereal Foods World 2001; 46:196-199.

Malkki Y, Virtanen E. Gastrointestinal effects of oat bran and oat gum - A review. Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology 2001; 34:337-347.

Mariadason JM, Catto-Smith A, Gibson PR. Modulation of distal colonic epithelial barrier function by dietary fibre in normal rats. Gut 1999; 44:394-399.

Marliss EB, Nakhooda AF, Poussier P. Clinical forms and natural history of the diabetic syndrome and insulin and glucagon secretion in the BB rat. Metabolism 1983; 32:11-17.

Mathieu C, Kuttler B, Waer M, Bouillon R, Hahn HJ. Spontaneous Reestablishment of Self-Tolerance in Bb/Pfd Rats. Transplantation 1994; 58:349-354.

Mattisson I, Wirfalt E, Johansson U, Gullberg B, Olsson H, Berglund G. Intakes of plant foods, fibre and fat and risk of breast cancer--a prospective study in the Malmo Diet and Cancer cohort. Br.J Cancer 2004; 90:122-127.

McCullough JS, Ratcliffe B, Mandir N, Carr KE, Goodlad RA. Dietary fibre and intestinal microflora: effects on intestinal morphometry and crypt branching. Gut 1998; 42:799-806.

Mcintosh GH, Whyte J, Mcarthur R, Nestel PJ. Barley and Wheat Foods - Influence on Plasma-Cholesterol Concentrations in Hypercholesterolemic Men. American Journal of Clinical Nutrition 1991; 53:1205-1209.

Mckay S, Ball M. Do beans and oat bran add to the effectiveness of a low-fat diet. European Journal of Clinical Nutrition 1992; 46:641-648.

McPherson R. Dietary fiber-a perspective. In: Spiller G, editor. Dietary Fiber in Human Nutrition. Boca Raton: CRC Press, 1993. p. 7-14.

Meddings JB, Jarand J, Urbanski SJ, Hardin J, Gall DG. Increased gastrointestinal permeability is an early lesion in the spontaneously diabetic BB rat. American Journal of Physiology-Gastrointestinal and Liver Physiology 1999; 276:G951-G957.

Mishima S, Xu DZ, Deitch EA. Increase in endotoxin-induced mucosal permeability is related to increased nitric oxide synthase activity using the Ussing chamber. Critical Care Medicine 1999; 27:880-886.

Mineo H, Amano M, Chiji H, Shigematsu N, Tomita F, Hara H. Indigestible disaccharides open tight junctions and enhance net calcium, magnesium, and zinc absorption in isolated rat small and large intestinal epithelium. Digestive Disease and Science 2004; 49:122-132.

Moore N, Chao CW, Yang LP, Storm H, Oliva-Hemker M, Saavedra JM. Effects of fructo-oligosaccharide-supplemented infant cereal: a double-blind, randomized trial. British Journal of Nutrition 2003; 90:581-587.

Morais MB, Vitolo MR, Aguirre ANC, Fagundes-Neto U. Measurement of low dietary fiber intake as a risk factor for chronic constipation in children. Journal of Pediatric Gastroenterology and Nutrition 1999; 29:132-135.

Mosenthal AC, Xu DZ, Deitch EA. Elemental and intravenous total parenteral nutrition diet-induced gut barrier failure is intestinal site specific and can be prevented by feeding nonfermentable fiber. Critical Care Medicine 2002; 30:396-402.

Nakhooda A, Like A, Chappel C, Murray F, Marliss E. The spontaneous diabetic wistar rats: metabolic and morphologic studies. Diabetes 1976; 26:415-417.

Ohta K, Omura K, Hirano K, Kanehira E, Ishikawa N, Kato Y, Kawakami K, Watanabe G. The effects of an additive small amount of a low residual diet against total parenteral nutrition-induced gut mucosal barrier. American Journal of Surgery 2003; 185:79-85.

Omura K, Hirano K, Kanehira E, Kaito K, Tamura M, Nishida S, Kawakami K, Watanabe Y. Small amount of low-residue diet with parenteral nutrition can prevent decreases in intestinal mucosal integrity. Annals of Surgery 2000; 231:112-118.

Ou S, Kwok K, Li Y, Fu L. In vitro study of possible role of dietary fiber in lowering postprandial serum glucose. J.Agric.Food Chem. 2001; 49:1026-1029.

Rehman KU, Wingertzahn MA, Teichberg S, Harper RG, Wapnir RA. Gum arabic (GA) modifies paracellular water and electrolyte transport in the small intestine. Digestive Disease Science 2003; 48:755-760.

Reimer RA, Field CJ, McBurney MI. Ontogenic changes in proglucagon mRNA in BB diabetes prone and normal rats weaned onto a chow diet. Diabetologia 1997; 40:871-878.

Reimer RA, Glen S, Field CJ, McBurney MI. Proglucagon and glucose transporter mRNA is altered by diet and disease susceptibility in 30-day-old biobreeding (BB) diabetes-prone and normal rats. Pediatric Research 1998; 44:68-73.

Reimer RA, McBurney MI. Dietary fiber modulates intestinal proglucagon messenger ribonucleic acid and postprandial secretion of glucagon-like peptide-1 and insulin in rats. Endocrinology 1996; 137:3948-3956.

Rodriguez-Cabezas ME, Galvez J, Camuesco D, Lorente MD, Concha A, Martinez-Augustin O, Redondo L, Zarzuelo A. Intestinal anti-inflammatory activity of dietary fiber (Plantago ovata seeds) in HLA-B27 transgenic rats. Clin.Nutr. 2003; 22:463-471.

Roediger W. The place of short-chain fatty acids in colonocyte metabolism in health and ulcerative colitis: the impaired coloncyte barrier. In: Cummings J, Rombeau J, Sakata T, editors. Physiological and clinical aspects of short-chain fatty acids. Cambridge: Cambridge University Press, 1995. p. 337-351.

Rubin B. Small intestine anatomy and structural anomalies. In: Yamada T, Alpers D, Kaplowitz W, Laine L, Owyang C, Powell D, editors. Textbook of Gastroenterology. Philadelphia: Lippincott Willamas & Wilkins, 2003. p. 1466-1485.

Sakata T. Effects of short-chain fatty acids on the proliferation of gut epithelial cells *in vivo*. In: Cummings J, Rombeau J, Sakata T, editors. Physiological and clinical aspects of short-chain fatty acids. Cambridge: Cambridge University Press, 1995. p. 289-305.

Schley PD, Field CJ. The immune-enhancing effects of dietary fibres and prebiotics. British Journal of Nutrition 2002; 87 Suppl 2:S221-S230.

Schneeman BO. Gastrointestinal physiology and functions. British Journal of Nutrition 2002; 88 Suppl 2:S159-S163.

Schwartz S, Levine GD, Starr C. Effects of dietary fiber on intestinal ion fluxes in rats. American Journal of Clinical Nutrition 1982; 36:1102-1105.

Shiau SY, Chang GW. Effects of Certain Dietary-Fibers on Apparent Permeability of the Rat Intestine. Journal of Nutrition 1986; 116:223-232.

Shiner M. Anatomy of the small intestine. In: Cohen S, Pounder R, Kalser M, Farmer R, Shape W, Bodenheimer H, Banks S, editors. Bockus Gastroenterology. Philadelphia: W.B. Saunders, 1994. p. 885-898.

Sigleo S, Jackson MJ, Vahouny GV. Effects of Dietary Fiber Constituents on Intestinal Morphology and Nutrient Transport. American Journal of Physiology 1984; 246:G34-G39.

Smecuol E, Bai JC, Sugai E, Pedreira S, Niveloni S, Vazquez H, Mazure R, Boerr L, Maurino E, Meddings J. Intestinal sucrose permeability in celiac disease (CD). Gastroenterology 1997; 112:A406.

Söderholm J. Epithelial barrier dysfunction in ileal Crohn's disease. 1-128. 1998. Faculty of Health Sciences, University of Linkoping.
Ref Type: Thesis/Dissertation

Southgate D. The definition, analysis and properties of dietary fibre. In: Heaton K, editor. Dietary Fibre: Current Developments of importance to health. Westport: Food & Nutrition Press, 1977. p. 9-19.

Spaeth G, Gottwald T, Specian RD, Mainous MR, Berg RD, Deitch EA. Secretory immunoglobulin A, intestinal mucin, and mucosal permeability in nutritionally induced bacterial translocation in rats. Ann.Surg. 1994a; 220:798-808.

Spiller G. Definition of dietary fiber. In: Spiller G, editor. Dietary Fiber in Human Nutrition. Boca Raton: CRC Press, 1993. p. 15-18.

Steward M, Case M. Principles of ion and water transport across epithelia. In: Davidson J, editor. Gastrointestinal Secretion. London: Wright, 1989. p. 1-69.

Stillie R. Dietary fermentable fibre affect immune function in BB rats, an animal model of type 1 diabetes. 1-131. 2003. University of Alberta.
Ref Type: Thesis/Dissertation

Sundberg B, Wood P, Lia A, Andersson H, Sandberg AS, Hallmans G, Aman P. Mixed-linked beta-glucan from breads of different cereals is partly degraded in the human ileostomy model. American Journal of Clinical Nutrition 1996; 64:878-885.

Tappy L, Gugolz E, Wursch P. Effects of breakfast cereals containing various amounts of beta-glucan fibers on plasma glucose and insulin responses in NIDDM subjects. Diabetes Care 1996; 19:831-834.

Tasman-Jones C, Owen RL, Jones AL. Semipurified dietary fiber and small-bowel morphology in rats. Digestive Disease Science 1982; 27:519-524.

Teichberg S, Mcgarvey E, Bayne MA, Lifshitz F. Altered Jejunal Macromolecular Barrier Induced by Alpha-Dihydroxy Deconjugated Bile-Salts. American Journal of Physiology 1983; 245:G122-G132.

Thomson ABR. Experimental diabetes and intestinal barriers to absorption. American Journal of Physiology 1983; 244:G151-G159.

Thomson ABR, Jarocka-Cyrta E, Faria J, Wild G. Small bowel review-part II. Canadian Journal of Gastroenterology 1997; 11:159-165.

Thulesen J, Hartmann B, Nielsen C, Holst JJ, Poulsen SS. Diabetic intestinal growth adaptation and glucagon-like peptide 2 in the rat: effects of dietary fibre. Gut 1999; 45:672-678.

TOMAS G. Paneth cells-guardians of the gut cell hatchery. Nature Immunology 2004; 1:99-100.

Torronen R, Kansanen L, Uusitupa M, Hanninen O, Myllymaki O, Harkonen H, Malkki Y. Effects of An Oat Bran Concentrate on Serum-Lipids in Free-Living Men with Mild to Moderate Hypercholesterolemia. European Journal of Clinical Nutrition 1992; 46:621-627.

Trowell H. dietary fibre: a paradigm. In: Trowell H, Burkitt D, Heaton K, editors. Dietary Fibre, Fibre-Depleted Foods and Disease. London: Academic Press, 1985. p. 1-20.

Trowell H, Burkitt D, Heaton K. Definitions of dietary fibre and fibre-depleted foods. In: Trowell H, Burkitt D, Heaton K, editors. Dietary Fibre, Fibre-Depleted Foods and Disease. London: Academic Press, 1985. p. 21-30.

Ussing H, Zerahn K. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. Acta Physiologica Scandinavica 1951; 23:111-127.

Vachon C, Jones JD, Wood PJ, Savoie L. Concentration Effect of Soluble Dietary-Fibers on Postprandial Glucose and Insulin in the Rat. Canadian Journal of Physiology and Pharmacology 1988; 66:801-806.

van Egmond M, Damen CA, van Spriel AB, Vidarsson G, van Garderen E, van de Winkel JGJ. IgA and the IgA Fc receptor. Trends in Immunology 2001; 22:205-211.

Vuksan V, Jenkins DJA, Spadafora P, Sievenpiper JL, Owen R, Vidgen E, Brighenti F, Josse R, Leiter LA, Bruce-Thompson C. Konjac-Mannan (glucomannan) improves glycemia and other associated risk factors for coronary heart disease in type 2 diabetes - A randomized controlled metabolic trial. Diabetes Care 1999; 22:913-919.

Vuksan V, Sievenpiper JL, Owen R, Swilley JA, Spadafora P, Jenkins DJ, Vidgen E, Brighenti F, Josse RG, Leiter LA, Xu Z, Novokmet R. Beneficial effects of viscous dietary fiber from Konjac-mannan in subjects with the insulin resistance syndrome: results of a controlled metabolic trial. Diabetes Care 2000; 23:9-14.

Walker J, Jijon HB, Churchill T, Kulka M, Madsen KL. Activation of AMP-activated protein kinase reduces cAMP-mediated epithelial chloride secretion. American Journal of Physiology-Gastrointestinal and Liver Physiology 2003; 285:G850-G860.

Wood PJ. Physiochemical properties and phsyiological effects of the (1–>3)(1–>4)-ß-D-glucan from oats. Adv Exp Med Biol 1990; 270:119-128.

Wood PJ, Beer MU, Butler G. Evaluation of role of concentration and molecular weight of oat beta-glucan in determining effect of viscosity on plasma glucose and insulin following an oral glucose load. British Journal of Nutrition 2000; 84:19-23.

Wood PJ, Braaten JT, Scott FW, Riedel KD, Wolynetz MS, Collins MW. Effect of Dose and Modification of Viscous Properties of Oat Gum on Plasma-Glucose and Insulin Following An Oral Glucose-Load. British Journal of Nutrition 1994; 72:731-743.

Wood PJ, Weisz J, Beer MU, Newman CW, Newman RK. Structure of (1 -> 3)(1 -> 4)-beta-D-glucan in waxy and nonwaxy barley. Cereal Chemistry 2003; 80:329-332.

Wood P. Physicochemical characteristics and physiological properties of oat (1–>3)(1–>4)-ß-D-glucans. In: Wood PJ, editor. Oat bran. St. Paul: American Association of Cereal Chemists, 1993. p. 83-112.

Wring SA, Terry A, Causon R, Jenner WN. The electroanalysis of mannitol, xylose and lactulose at copper electrodes: voltammetric studies and bioanalysis in human urine by means of HPLC with electrochemical detection. Journal of Pharmaceutical and Biomedical Analysis 1998; 16:1213-1224.

Wursch P, PiSunyer FX. The role of viscous soluble fiber in the metabolic control of diabetes - A review with special emphasis on cereals rich in beta-glucan. Diabetes Care 1997; 20:1774-1780.

Yang H, Finaly R, Teitelbaum DH. Alteration in epithelial permeability and ion transport in a mouse model of total parenteral nutrition. Critical Care Medicine 2003; 31:1118-1125.